

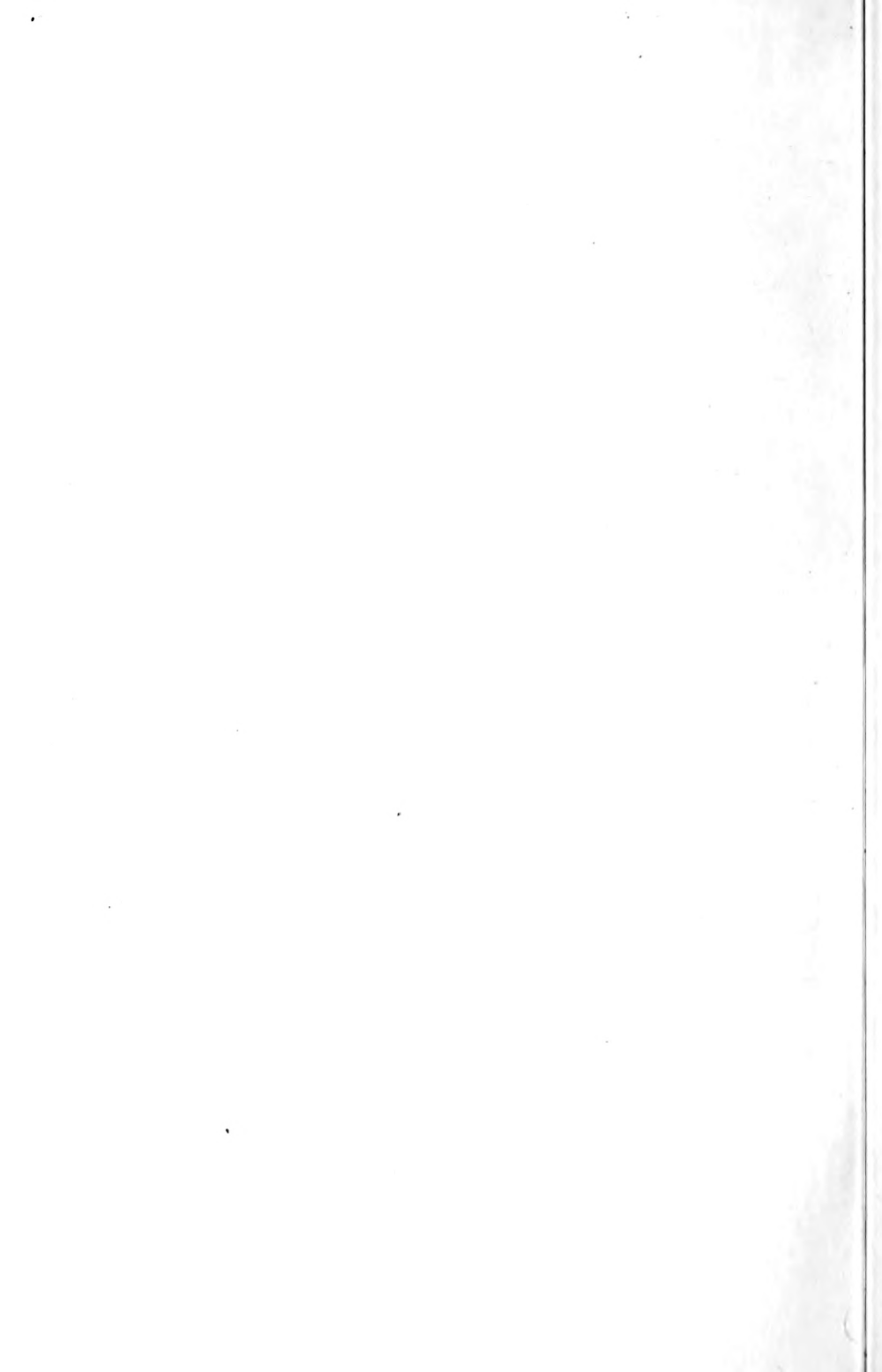
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Development and Origin of the Respiratory Organs in Araneæ.

25

By

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 With Plates 1—7 and 7 Text-figures.

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I. INTRODUCTION.

It is just one hundred years ago that the first anatomical account of the lung-books of Arachnida was published by Meckel ('09), who, like his immediate successors, looked upon these organs as gills, and it was not until 1828 that their pulmonary nature was recognised by Johannes Müller ('28a, '28b) and Straus-Durckheim ('28). The latter was also, I be-

lieve, the first to point out (p. 315) that the lung-books could be regarded as a special form of tracheæ, a view which was later on elaborated by Leuckart ('48, p. 119 note, and '49) and for a time generally accepted, until the appearance of Ray Lankester's paper, "*Limulus: an Arachnid*," in 1881, opened up the probability of the branchial origin of these organs.

While working at certain points in the embryology of a spider some years ago it occurred to me that a more careful and detailed investigation of the development of the lung-books and tracheæ than had hitherto been attempted would probably reveal some points of interest in connection with the origin of these organs, and indeed it soon appeared that two important facts had been entirely overlooked, viz. (1) the appearance of the earliest lung-leaves on the free posterior side of the provisional abdominal appendages quite outside of the pulmonary invagination, and (2) the origin of a considerable part of the tracheæ from ectodermal tendons (entapophyses) and not from lung-books. This latter appeared to me a point of particular interest, as it is the only case, I believe, in which the origin of a trachea from another organ not respiratory in nature can be clearly demonstrated.

My investigations were carried out in the years 1894 and 1895, in the Zoological Laboratory of the University at Berlin, and my thanks are due to Geheimrath Prof. F. E. Schulze for the use of his splendidly equipped laboratory. About one third of the text had already been written and most of the figures drawn when I left Berlin in 1895 for South Africa, where various circumstances prevented the completion of the paper for the press until quite recently.

Material.—The material for the development was collected in the neighbourhood of Berlin, and consisted of the embryos and young of *Sitticus* (*Attus*¹) *floricola* C. K., of which I had an unlimited supply of all the required stages of development. Besides these I examined a small number of

¹ This name has been recently discarded by E. Simon and *Sitticus* substituted in its stead.

embryos and young of *Agelena labyrinthica* and of *Tegenaria atrica*, but the account of the embryology in the following pages applies only to *Attus floricola*, unless the contrary is expressly stated.

The material required for anatomical purposes consisted of adult or subadult specimens of forty-one species mostly obtained in the neighbourhoods of Berlin or Cape Town, as stated in the list given below. The specific determination of the European specimens (except *Tegenaria atrica*) were made from Dahl ('83), but the families and genera are in agreement with E. Simon ('Hist. Nat. Araign.,' 2nd ed.).

LIST OF THE SPECIES USED.

(The twenty-nine species marked with an asterisk [*] were also examined in sections.)

Tetrapneumonous Spiders.

Fam. Aviculariidae.

Sub-fam. Aviculariinae.

**Crypsidromus intermedius*, Paraguay.

Harpactira atra, Latr., Cape Town.

Sub-fam. Ctenizinae.

Stasimopus unispinosus, Purc., Cape Colony.

Hermacha sp., Cape Town.

Dipneumonous and Apneumonous (Caponia) Spiders.

Fam. Eresidae.

Eresus sp., Cape Town.

Fam. Sicariidae.

**Scytodes testudo*, Purc., Cape Town.

Fam. Dysderidae.

**Dysdera* sp., Berlin.

**Harpactes Hombergi*, Scop., Berlin.

**Segestria senoculata*, L., Berlin.

Fam. Caponiidæ.

**Caponia spiralis*, Purc., Cape Colony.

Fam. Drassidæ.

**Drassodes* (*Drassus*) *infuscatus*, Westr., Berlin.
D. tessellatus, Purc., Cape Colony.

**Melanophora* (*Prothesima*) *Petiveri*, Scop.,
Berlin.

Fam. Palpimanidæ.

**Palpimanus* sp., Cape Town.

Fam. Theridiidæ.

Latrodectus geometricus, C. K., Cape Town.

**Theridion lineatum*, Cl., Berlin.

Fam. Argiopidæ.

Sub-fam. Linyphiinæ.

**Linyphia triangularis*, Cl., Berlin.

Sub-fam. Tetragnathinæ.

**Pachygnatha Listeri*, Sund., Berlin.

Sub-fam. Nephilinae.

Nephila sp., Senegal.

Sub-fam. Argiopinae.

Argiope clathrata, C. K., Cape Town.

Fam. Thomisidæ.

**Philodromus* (*Artanes*) *fuscomarginatus*, De
G., Berlin.

**P.* (*Artanes*) *pallidus*, Walck., Berlin.

**Tibellus oblongus*, Walck.

Fam. Clubionidæ.

Palystes sp., Cape Town.

**Clubiona holosericea*, De G., Berlin.

**Zora* sp., Berlin.

Fam. Agelenidæ.

**Argyroneta aquatica*, Cl., Berlin.

**Textrix lycosina*, Sund., Berlin.

**Agelena labyrinthica*, Cl., Berlin.

**Tegenaria atrica*, C. K., Berlin.

T. domestica, Cl., Cape Town.

Fam. Pisauridæ.

**Pisaura* (*Ocyale*) *mirabilis*, Cl., Berlin.

**Dolomedes* sp., Berlin.

Fam. Lycosidæ.

**Lycosa* (*Trochosa*) sp., Berlin.

**L.* (*Pirata*) *hygrophila*, Thor., Berlin.

**L.* (*Tarantula*) *aculeata*, Cl., Berlin.

L. Darlingi, Poc., Cape Town.

**L.* sp., Berlin.

Fam. Salticidæ (*Attidæ*).

**Sitticus* (*Attus*) *floricola*, C. K., Berlin.

S. (Attus) sp., Berlin.

**Marpissa* (*Marpessa*) *mucosa*, Cl., Berlin.

Biological observations.—*Attus floricola* fastens its cocoons on dead branches, etc., on the edges of the lakes in the Grunewald, a forest near Berlin, and I have found as many as twenty or thirty cocoons closely packed together in a group at the N.W. corner of Hundekühle See. The number of eggs in a cocoon varies normally from about thirty-five to fifty, and eggs may be found in the cocoons throughout June, July, and the first half of August.

A number laid in captivity on July 12th hatched (i. e. burst the egg-shell) on July 28th and 29th, i. e. after sixteen to seventeen days. At the time of hatching the embryos are still very imperfectly formed and very much resemble Locy's fig. 10, except that the legs, which are curved inwards and ventrally, are segmented. The pedipalps are each provided at the base with a small conical tooth, which is broader than high and drawn out at its apex into a tiny brown point. Shortly before hatching the egg-shell becomes stretched and raised on the tips of the two teeth, which then split it across in front of the chelicera.¹

¹ I also found the two teeth in *Xysticus*, *Tegenaria*, and *Agelena*, the teeth and the part of the cuticula on which they stand being black in the two latter genera. These teeth do not appear to have been previously observed, and they have been recorded in Korschelt and

After hatching the embryos remain motionless for five to six days or even a little longer before the first post-embryonic moult takes place, after which the young spiders acquire the use of their limbs. They are still, however, in a very imperfect condition, especially as regards the eyes. They remain in the cocoons until after the second moult, which takes place sixteen to seventeen days after the first. The young spiders then emerge in a perfect condition, with fully-developed eyes, and have also acquired the definite shape of the adult.¹

The entire development, therefore, takes from about thirty-seven to forty days, less than half of which number is spent within the egg-shell.

Treatment.—The preserving reagent upon which I mostly relied was a hot concentrated alcoholic solution of corrosive sublimate, which I make use of in the following manner: A quantity of sublimate is placed in a small, loosely corked, boiling flask with some alcohol of 70 per cent. and heated over a flame with constant shaking until the alcohol begins to boil. Some of the concentrated solution is then poured into a small tube of about 3 c.cm. capacity, which is immediately corked and suspended by a string in a basin of water heated to 80° C.² A few eggs are now dropped in and removed almost immediately afterwards by means of a thin glass rod, which is flattened at one end and here bent at right angles to form a scoop large enough to take out one egg at a time.³ The sublimate will probably be precipitated during the latter operation, but that does not matter. The eggs are then placed in 63 per cent. alcohol (70 per cent. will

Heider ('92, p. 588). Later on I found a similar black tooth on the base of each pedipalp in the embryo of a Tetrapneumonous spider (*Harpactira atra*) from Cape Town.

¹ Similar phases occur in the development of all other Dipneumonous spiders which I have examined.

² A temperature of 70° has much the same effect.

³ It sometimes happens that the egg-shell bursts, in which case the embryo is destroyed by the violent action of the reagent. As a rule, however, it remains intact and only just sufficient reagent penetrates to preserve the embryo.

do as well) for one hour, the alcohol being changed several times, then for one to two hours in 78 per cent. alcohol, and finally in 93 per cent. and absolute alcohol. Chloroform was used for embedding in paraffin and the sections were stained on the slide in alum carmin, which I found the most suitable for them.

A number of embryos were also preserved in a hot aqueous solution of corrosive sublimate (concentrated at the ordinary temperature) for the purpose of control. I did not, however, require to make much use of these embryos, which could not compare with the others in point of preservation.

The great disadvantage attached to the use of plain hot water or hot aqueous solutions, which are the methods hitherto usually employed in spider embryology, lies in the circumstance that the embryonic tissues take the appearance of a syncytium when so preserved. It accordingly becomes difficult to distinguish between two or more epithelia in contact, the several layers of nuclei being then usually the only feature by which the nature of the tissue can be guessed at rather than recognised.

I devised the hot alcoholic sublimate method described above only after repeated experiments. It has the merit of rendering the contours of the cells much more distinct, so that not only the boundary line between two epithelia in contact, but generally also the boundary lines between adjacent cells within an epithelium becomes recognisable.

In the sections these cell-contours may appear in the form of fine dark lines or they may be indicated merely by a difference in the staining between the protoplasm of adjoining cells or by the presence of paler lines between the cells, as if these had been slightly drawn apart,

As the appearance of the protoplasm was of minor importance, but the contours of the epithelia and their cells of the greatest importance in my investigations, I have for convenience represented the contours in the figures by dark lines, by which, of course, I do not intend to imply that regular cell-walls are present.

I found it quite impossible to obtain an accurate idea of the rudimentary lung-books and tracheæ in the embryos, except by means of reconstructions, of which extensive use was made. For the complicated lung-books a large number of the ordinary reconstructions with wax tablets were made (thickness of sections 5.82μ , of wax tablets 2 mm.; magnified 343.7 diameters), but for the simpler tracheæ the following method was employed:

A sheet of transparent paper is placed over another of white paper ruled with a series of parallel lines 2 mm. apart. The width of the organ to be reconstructed magnified the required number of times (343.7 times for sections 5.82μ thick), is measured with a pair of compasses in each section and marked off on the parallel lines, each of which represents a section. When all the sections have been marked in this way on the transparent paper the outlines of the organ will be obtained in their correct proportions. This method is much quicker than the other and very suitable for reconstruction in outline from transverse sections of any bilaterally symmetrical organ of simple form, such as the embryonic tracheæ in the later stages (figs. 28 and 29). By drawing a line down the middle of the paper at right angles to the parallel lines to represent the median line of the body, and marking each transverse section symmetrically on each side of this line, the symmetry of the reconstructed organ will be preserved.

II. GENERAL ORIENTATION.

Lung-books.—A typical well-developed lung-book of a Dipneumonous spider has the following parts (figs. 20 and 21):

(1) A more or less transverse spiracle (*sp.*) or stigma placed laterally at the junction of the ventral and lateral surfaces of the second abdominal segment along its hind margin (text-fig. 1).

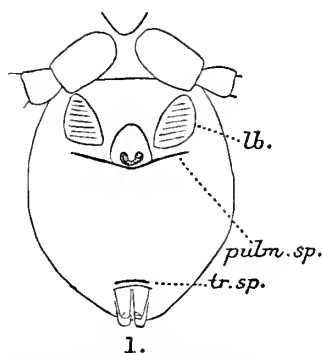
(2) A short flattened tube leading forwards from the spiracle into the body in a slightly upward and medial direc-

tion, forming a stalk or pedicel (*ped.*) to the whole lung-complex. This opens into—

(3) An elongated-lanceolate hollow band, the pulmonary sac (ante-chamber or vestibule, *pulm. s.*), which runs from just in front of the medial angle of the spiracle at first in a dorso-lateral direction, but becomes procurved at a greater or less distance beyond the lateral angle of the spiracle to form the horn (Schneider, *h.*) and terminates in a short, blind, apical pouch (*ap.*).

(4) A series of long, flattened, hollow pouches (sacculi, *s.*), which are triangular in shape, like a flattened butterfly-net, generally horizontal, and placed one over the other in a

TEXT-FIG. 1.



Attus floricola. Ventral surface of abdomen. *lb.* Pulmonary operculum. *pulm. sp.* Pulmonary spiracle. *tr. sp.* Tracheal spiracle. Magnified 13.

slightly imbricating manner (each being slightly more lateral than the one below it), like the leaves of an open book. The sacculi, being invaginations of the anterior wall of the ante-chamber, communicate with its lumen by their open posterior ends, which form a series of parallel slits, like an oven-grate (Bertkan), extending obliquely across the entire anterior surface of the ante-chamber, including the corresponding ventral surface of the procurved horn, being absent only from the small apical pouch of the latter.¹

¹ In some text-books, e. g. Korschelt and Heider ('92, p. 605, fig. 382)

All these parts, being hollow, contain air in direct communication with the external atmosphere. The partition walls between the air-spaces of adjacent saccules, I shall term the "septa."¹ The dorsal side of each septum is studded with numerous, simple, blunted spines, which keep the lumens of the saccules open, while the walls of the ante-chamber (including its fenestrated anterior wall and the apical pouch of the horn), are covered with peculiar hooped spines (spines with anastomosing apical branches). The pedicel is for the most part unspined.

The two spiracles are generally united by a transverse fold, the epigastric or interpulmonary fold (*interp. fld.*), which also connects the two pedicels and the extreme medial corners of the two ante-chambers (see text-fig. 1). The lumens of the latter at the same time communicate by the interpulmonary canal of communication (*can.*), or passage with hooped spines in the upper edge of the fold.

Further remarks on the lung-books of the adult are given on p. 41, and an historical account of the literature will be found at the end of the paper.

Tracheæ.—The usual form of tracheæ in a Dipneumonous spider has the following parts (figs. 21, 25 and 31):

(1) A median, transverse, ventral spiracle (*sp.*), placed on the hind margin of the third abdominal segment usually just before the spinners (text-fig. 1).

the procurved horn is wrongly represented as having no saccules opening into it.

¹ In order to avoid ambiguity I have substituted the terms "sacculi" and "septa" in place of the old terms "leaves" and "lamellæ." The older writers almost invariably meant to indicate the saccules when they used the term "leaves" (*feuillet*, Blatter), but since about 1881 the term has generally been employed for the septa, like the term "lamellæ." Neither term, however, has at present any definitely recognised use. Thus "lamelles" signifies the septa with MacLeod ('84), but only one of the layers of a septum with Berteaux ('89), whose term for a whole septum is "lame," while "feuillet" signifies a septum with Schimkewitsch ('84) and Plateau ('86), but a sacculus with Schneider ('92).

(2) A short, flattened chamber (vestibule, *vest.*), leading forwards and upwards from the spiracle into the body and giving off at its anterior or deepest part—

(3) A pair of medial (*m.tr.*) and a pair of lateral tracheal trunks (*l.tr.*), which may again give rise to tracheal branches (*br.*, fig. 21), the finest of these being the tracheal tubules (*tr.tub.*, fig. 31). The trunks and branches are lined with anastomosing spines (more rarely with spiral threads only), but the fine tubules have only spiral threads.

The anterior or deepest part of the cavity of the vestibule is always widened to form a transverse canal of communication (*can.*) or passage with hooped spines, connecting the cavities of the tracheal trunks. The remaining or smooth portion of the vestibule forms a stalk or pedicel (*ped.*) to the whole tracheal system, and is supported on each side by chitinous thickening or rod (*rd.*).

III. HISTORICAL (DEVELOPMENT).

Development of the lung-books in Arachnida.—Metschnikoff ('71) gives an account of the development of the lung-books in scorpions, and observes that they arise as ectodermal invaginations just behind the four posterior pairs of abdominal appendages, which latter subsequently atrophy. Towards the end of the embryonic period the folds in the pulmonary sacs appear.

Salensky ('71) was the first to study their development in Araneæ, and believed that the lung-books were formed by the invagination of the abdominal appendages (teste Jaworowski, '94, p. 55).

Bertkau ('72) showed that in the young spider, after the completion of the embryonic period, the lung-books continue to develop, new leaves being added at the growing dorso-lateral end, each new leaflet arising next to the one previously formed.

Locy ('86) gives a detailed description of the later stages

in a spider (*Agelena nævia*), and he is the first to give an account of the transformation of the embryonic epithelial foldings into the definite pulmonary septa (lamellæ) with their chitinous coverings. According to him the lung-books arise as a pair of invaginations late in the period of the reversion, but he makes no mention of their connection with appendages.

Bruce ('86a, '86b, '87) is of opinion that the pulmonary folds in spiders are formed on the anterior surface of the first abdominal appendage, which subsequently becomes involuted, so that its anterior surface with the folds now faces the posterior end. Probably two abdominal appendages are invaginated for each lung-book.

Schminkewitsch ('87; also in '86a and '86b, teste Jaworowski, '94) states that the lung-book arises as an invagination of the ectoderm and forms a true trachea, consisting of a main trunk divided into five branches, in the embryo of *Lycosa saccata* just before hatching. Recently, however, Schminkewitsch ('06, pp. 45, 46, footnote) has withdrawn this interpretation.

Kowalevsky and Schulgin ('86) merely note that the pulmonary sacs in the scorpion (*Androctonus ornatus*) arise as simple invaginations into a space containing plenty of blood.

Morin ('87) found that the lung-books in the spider (*Theridion*) arise from a pair of ectodermal invaginations at the base of the first pair of abdominal appendages, which themselves become the lung-opercula. In his later paper ('88) he appears to have given more details of the formation of the lamellæ, of which those nearest the operculum are furthest developed (teste Jaworowski '94, pp. 57 and 58).

Laurie ('90) states that in the scorpion (*Euscorpium italicus*) the four last pairs of abdominal appendages are pushed in on their posterior part, so as to form shallow, cup-like cavities, which later on are divided up by lamellæ growing down from their upper ends (pp. 125 and 127). A later stage with lamellæ is also described (p. 129). In a later paper ('92) he deals with the development in *Scorpio fulvipes*.

Kishinouye ('90) confirms the statement regarding the lung-books and the opercula contained in Morin's earlier paper ('87), and adds that that wall of the invagination which faces the distal end of the appendage is much thickened, filling the interior of the appendage, the cells becoming after a while arranged in parallel rows to form the septa. He examined *Lycosa*, *Agelena*, *Theridion*, *Epeira*, *Dolomedes* and *Pholcus*.

Simmons' ('94) paper is the most important that has yet appeared on the development of the spider's lung-books, and was based on embryos of *Agelena naevia* and *Theridion tepidarium*. He confirms Morin's and Kishinouye's statements regarding the formation of the pulmonary invagination, and was the first to describe and figure an early stage of the formation of the pulmonary septa (lamellæ), which he states arise as infoldings upon the posterior surface of the abdominal appendage in the same manner as described by Kingsley for the gills of *Limulus*.

Jaworowski ('93 and '94) describes the presence in a spider embryo (*Trochosa*) of embryonic tracheæ, which ultimately becomes rudimentary, excepting the portion adjoining the spiracle. The wall of this portion is thrown into folds and persists as the lung-books. The author thus totally differs in some most important points from all his predecessors. These tracheæ arise from invaginations under the abdominal appendages, the latter becoming the opercula ('95, p. 43). Jaworowski also gives a valuable account of the formation of the definite pulmonary septa out of the folded embryonic epithelia.

According to Laurie ('94) the embryonic abdominal appendages are not paired in the Pedipalpi, but stretch right across the abdomen, and in *Phrynus* the lung-books evidently arise as foldings of the posterior wall of an appendage.

Brauer ('95) confirms Metschnikoff's and Laurie's observations on the earlier stages of the pulmonary invaginations at the base of the four posterior pairs of abdominal appendages

in scorpions, and gives the best account of the early stages of the pulmonary folds (in *Euscorpius carpathicus*).

In my own paper ('95) the appearance of the earliest pulmonary folds on the free posterior side of the first pair of abdominal appendages in *Araneæ* is described.

Sophie Pereyaslawzewa (:01) investigated the earliest appearance of the lung-books in *Phrynidæ* in *Phryniscus bacillifer* and the later stages in *Damon medius*. According to her the lung-books are formed from the third and fourth abdominal appendages, which belong to the third and fourth abdominal segments (p. 194). The outer integument, the cuticula of which is regularly wrinkled (fig. 61), is deeply infolded into the body behind the third and fourth appendages to form the lung-sacs, the grooves in the invaginated wrinkled surface deepening to form the sacculi, while the ridges become the septa (pp. 248-252). The embryonic septa are also described (p. 262) and figured (fig. 69).

Gough (:02) states that the lung-books in an embryo of a Pedipalp (*Phrynid*) belong to the first and second abdominal appendages. The author gives no further account of the development of the lung-books, but merely states that it does not differ from that in other *Arachnids* (p. 616).

Schinkewitsch (:03, :06) gives a more detailed account of the development of the lung-books in *Thelyphonus caudatus*. According to him the lung-books are formed from pulmonary sacs or invaginations at the base of appendages, which are placed on the hind margins of the second and third abdominal somites. The lung-leaves arise as folds in the lower wall of this sac, and later on the leaves, which were formed in the sac, come to lie outside of it on the posterior side of the appendages so that the sacculi then open to the outside instead of into the sac (fig. 46). Several sections of later stages of the lamellæ are figured.

Sophie Pereyaslawzewa (:07), in a posthumous memoir, describes and figures some interesting stages in the development of the lung-books of a scorpion (*Androctonus ornatus*) from the material left by Kowalevsky and Schulgin.

According to her the invagination which forms the pulmonary sac is situated on the anterior edge of the lateral part of the base of an abdominal appendage and is apparently unconnected with the latter (p. 177).

Development of the tracheæ in Araneæ.—Schinkewitsch ('87) states that the tracheæ in *Lycosa saccata* arise by invaginations of the ectoderm. In his Russian paper ('86a) he gives a figure of a developing trachea (*ect*, fig. 29A), without, however, recognising it as such.

Kishinouye ('90) observed an ectodermal invagination in various spiders in the basal part of the second abdominal appendage on the interior side. This invagination forms a deep tube at the time of hatching and the author calls it an "abortive trachea."

Simmons ('94) found the same invagination in *Agelena nævia* and *Theridion tepidariorum*, and in addition what he considers to be aborted lung-leaves.

Finally, in my abstract ('95) of the present paper the origin of the greater part of the tracheæ in *Attus floricola* from ectodermal tendons is stated in outline.

IV. THE PROVISIONAL ABDOMINAL APPENDAGES IN THE EMBRYO OF *ATTUS FLORICOLA*.

The description begins with the stage¹ immediately preceding the appearance of the pulmonary folds (stage 1, *St.* 1). The embryonic band has attained its greatest length, and the process known as the reversion is about to commence. A sagittal section (Pl. 1, fig. 4) through the abdominal region shows eight abdominal segments with coelomic sacs. The first abdominal (seventh post-oral) segment bears no appendages in this species, but the following four (eighth to eleventh post-oral) are each provided in their posterior region with a low, flat-topped,

¹ Corresponding to the stage in Korschelt and Heider, p. 581, fig. 369, and to Loey's Pl. ii, fig. 8, and Balfour's fig. 6. A list of the various stages and of the figures referring to each is given in the explanation of the plates.

provisional appendage (*ab. app.* 1-4) in successive stages of growth, that of the eleventh segment being the smallest and that of the eighth the largest.

The segments are marked off from each other by distinct transverse grooves, which are shallow, except immediately behind the appendages, where they are considerably deepened (*gr.*), and where the ectoderm forms a distinct post-appendicular fold, projecting at right angles, or nearly so, to the general surface into the body. The posterior wall of this fold is comparatively thin, like the adjacent epithelium of the following segment, but the anterior wall is much thicker, being, in fact, a direct continuation and a part of the posterior wall of an appendage, as I shall presently show.

A similar post-appendicular infolding (as distinct from the pulmonary sac to be described later) appears to be also found in *Limulus* (Kingsley, '85). In the older spider-embryo those of the posterior pairs of appendages serve as places of attachment for the ventral longitudinal muscles of the abdomen.

The deep infoldings behind the first pair of abdominal appendages extend from the medial end of the hind margin of each appendage nearly, but not quite, up to the extreme lateral end, and, moreover, the lateral part of the infolding (*gr.*, fig. 7A) is always slightly, but distinctly, deeper than its medial part (*gr.*, fig. 7). These two figures represent the appendages just before the earliest appearance of the rudiments of the lung-books.

V. THE DEVELOPMENT OF THE LUNG-BOOKS.

Stage with two pulmonary furrows (stage 2, *St.* 2).—The appendages of the pulmonary or eighth post-oral segment undergo considerable changes in passing from the stage just described to the next one, which I shall term the "stage with two pulmonary furrows." Fig. 1 is a transverse section of this stage, and shows that the appendages are still near together, although the reversion has commenced. This stage

follows so quickly upon the last that it is at first very puzzling to make out the changes accurately, but with the aid of numerous reconstructions in wax I have been able to ascertain the more important phases with certainty.

Fig. 14 is a sketch made from such a reconstruction, and represents the typical appearance of the right appendage seen somewhat from behind. Its distal surface is flat and often, although not always, distinctly transverse. Measured at the base, however, the breadth of the appendage is about equal to the antero-posterior diameter, and remains in this relation throughout the later stages. Seen from the distal surface the appendage appears distinctly four-sided, with its posterior side placed transversely to the embryonic band.

Fig. 8 is another reconstruction made from a series of longitudinal sections cut parallel to the principal axis (*pr. ax.*, fig. 1) of the appendage, and a number of sections from this series are given in figs. 8A-8H, the positions of the sections being indicated by the vertical lines in fig. 8.

The first point to be noticed is the subsidence of the epithelium (*ep.*, figs. 8A-8G) lying immediately behind the first abdominal appendage and forming the posterior lip of the post-appendicular groove¹ (*gr.*, in stage 1, fig. 7). The two lips of the latter thus become drawn completely apart along its whole length, so as almost to obliterate the groove as such (except at a single place to be mentioned presently) and lay free the whole posterior side of the appendage. In its median half the former bottom of the groove is now indicated only by a shallow furrow (*gr.*, figs. 8A-8D), which at the same time marks what in the previous stage (fig. 7) was the base of the posterior side of the appendage. This shallow furrow behind which the subsidence was greatest is more or less curved owing to a shifting backwards of the tissue in which it lies (*gr.*, fig. 14), so that the posterior side of the appendage comes to slant in its medial part at base (*sl.*, figs. 8A-8D,

¹ A corresponding subsidence also takes place anteriorly to the first appendage, causing the obliteration of the groove between the seventh and eighth segments.

and 14) much more than was the case in the previous stage, where the groove was straight and transverse. The angle of the slanting surface varies, the latter being in some embryos nearly perpendicular, in others nearly parallel to the adjacent body surface (*ep.*), and in the latter case the curved furrow may entirely disappear. The above will become clear by comparing figs. 8b and 9 of this stage with the corresponding section (fig. 7) of the previous stage.

In the second place we notice a little pocket-like cavity (*pulm. s.*, figs. 8 and 14) extending from the middle of the base behind in a lateral direction for about one third of the breadth of the appendage. This cavity, which we may term the pulmonary sac, is practically all that remains of the once extensive post-appendicular groove, and is to be considered as a portion of the latter which had become especially deepened and so escaped the obliteration which befel the rest of the groove—for a subsidence has also taken place in the tissue immediately behind the pocket. (Compare fig. 8g with the corresponding section, fig. 7A, of the previous stage.)

The pulmonary sac was first described and figured by Metschnikoff ('71) for the scorpion and was found by most subsequent investigators, but generally in a later stage of development.

The cells which form the wall of the sac undergo from now on repeated division (fig. 8g), causing the sac to grow rapidly, at first in a forward direction in the form of an in-pushing under the appendage, but later on in a latero-dorsal or dorsal direction (fig. 16). The anterior wall of the pulmonary sac yields the cell-material for all the lung sacculles, except the first two, whose appearance forms the third and most important point to be noticed in this stage.

On the medial half of the posterior side of the appendage there appear two parallel furrows of varying length (*f. 1, f. 2*, figs. 8 and 14). These are the first beginnings of the two oldest sacculles of the lung-book. They are never transverse but always incline to the longitudinal axis of the appendage

at varying angles. The first or medial furrow (*f. 1*) is always much the deeper and extends from near the medio-posterior angle of the base of the appendage in a latero-distal direction. As a rule when the posterior face of the appendage is strongly inclined, the furrow takes a more transverse direction and does not then reach the distal surface (as in figs. 8 and 14), but when the posterior face is less inclined, the furrow takes a direction more nearly parallel to the axis of the appendage, extends right up to the distal surface of the latter, and comes to be situated on its medio-posterior corner (fig. 10). In such cases, in fact, it is sometimes more on the medial than on the posterior side of the appendage. The second furrow (*f. 2*) appears almost simultaneously with the first, and is situated between the latter and the base of the appendage, so that its medial end terminates on the proximal side of the lateral part of the first furrow. It never extends right to the base nor to the distal surface of the appendage, and if produced medially would run proximally to the first furrow.

Compared with the preceding stage the medial half of the appendage has developed considerably and is sharply set off from the body surface. Further, in its lateral part (fig. 8g) the anterior side has become much more inclined than in the preceding stage (fig. 7A), so as to be parallel to the slanting anterior wall of the pulmonary sac. In longitudinal sections through this part (fig. 8g) the appendage has the false appearance of being directed backwards, and this becomes still more marked in later stages (as for instance figs. 12 and 16c). That this appearance is deceptive and merely due to the pulmonary sac will be readily seen if it be remembered that fig. 8g is a section lying between the sections fig. 8f and fig. 8h, and fig. 16c a section between fig. 16a and fig. 16d. The main axis of the appendage remains in all cases at right angles to the body.

Stages with three or more pulmonary furrows (stages 3 to 5).—The third furrow (*f. 3*, fig. 16) appears at the middle of the base of the posterior side of the appendage. It is

parallel to the others and lies partly inside and partly outside of the pulmonary sac. Its medial part lies proximally to the lateral end of the second furrow, and, in some cases at least, is continuous with the lateral end of the curved furrow mentioned above (*gr.*, fig. 14), which limits the appendage posteriorly. The fourth furrow (*f.* 4, fig. 16) and all subsequent ones lie wholly within the pulmonary sac and appear successively as oblique grooves in its anterior wall, all more or less parallel to those already formed and with the medial ends of each lying on the proximal side of the lateral part of the previously formed furrow.

After the appearance of the first two furrows the appendages rapidly move from a ventral to a lateral position owing to the reversion of the germinal band, and it is necessary to bear in mind that we must substitute the terms "dorsal" and "ventral" for "lateral" and "medial" after the lateral position has been reached.¹

Figs. 1-3 will make this clear. Fig. 1 is the position at the end of the 2-furrow stage; fig. 2 that at the end of the 3-furrow stage, and fig. 3 represents the position from the end of the 4-furrow stage, and here the appendages remain till near the close of the embryonic period. The whole segment which bears the appendage participates in this wandering, and the position of the appendage relatively to the adjacent surface is, of course, not affected by the movement.

It will be observed that the youngest furrow (fig. 3) is the most dorsal one, and, if produced, would lie on the proximal side of all the older ones.

The pulmonary sac increases hand in hand with the formation of new furrows, almost filling out the dorsal part of the hollow of the appendage. At the 5-furrow stage its blind end grows as a tube with a considerable lumen in an upward or dorsal direction, raising up the outer epithelium as it pushes its way underneath (see figs. 16, 16D, 16E).

¹ For the sake of uniformity and in order to facilitate comparison between them, the sections of the earlier and later stages of the appendages have been drawn in the same positions throughout.

Formation of the spiracle.—After the appendage has attained its greatest elevation (generally late in the 3-furrow stage) the whole region between the three oldest furrows begins to sink below the level of the appendicular posterior surface by a forward movement, causing it to be over-topped by the distal edge of the appendage (fig. 16A). This sinking movement, which must not be confounded with the formation of the pulmonary folds described further on, commences next to the pulmonary sac, and the latter thus comes to include first the third furrow (fig. 16B), then the second, and finally the first, while the common opening becomes the spiracle (*sp.*, fig. 13, which compare with figs. 13A and 13B of the same series).

Meanwhile that portion of the body epithelium which lies immediately in front of each of the four appendages in a row becomes absorbed into the anterior side of the appendage (compare figs. 4, 5, and 6), so that the four appendages appear closer together, while the original opening of the pulmonary sac comes to lie at the bottom of the groove so formed between the first and second appendages.

The lateral (afterwards dorsal) end of the spiracle is the first to be formed, and is already clearly defined immediately after the appearance of the pulmonary sac (fig. 14). The progressive development of the lateral part of the spiracle may be followed in figs. 8G, 12, and 16C. In the latter embryo the surface posterior (fig. 16C) and dorsal (fig. 16D) to the lateral end of the spiracle is almost on a level with the distal surface of the first appendage. The medial (later ventral) region of the spiracle remains open and undefined for a much longer time.

Sinking of the appendage.—This is a very simple process and begins about the 5-6-furrow stage (*St.* 5). The anterior and ventral sides become more slanting, so as to pass, like the dorsal side, more and more gradually over into the adjacent body surface, while the appendage itself decreases in elevation and sinks gradually into the body, until finally only a slight convexity in front of the spiracle marks its former position. (Compare fig. 13B, with five pulmonary furrows,

with the corresponding section, fig. 17, of a much later stage.)

Formation of the pulmonary saccules.—Next to their position on the posterior side of the appendage, the precise manner in which the saccules begin to form is the point of greatest interest and importance, when considered with regard to their possible direct origin from gill-lamellæ. I have been successful in obtaining a number of excellent sections through the region in question, showing the cell-boundaries with perfect distinctness. The position of these and of the nuclei of each individual cell have been drawn with the aid of a drawing apparatus in the sections figured in the plates, which are in this respect exact reproductions of the original sections (see p. 8).

The three figures 7, 9, and 8c represent longitudinal sections, cut parallel to the axis of the appendage, and, as nearly as possible, through the same region of the latter, in each case indicated by the line marked (*fig. 8c*) in fig. 8. All these sections are through the region in which the first furrow appears, and represent three consecutive phases following close upon one another.

In the youngest stage (fig. 7) no trace of the furrow is apparent, and the appendicular epithelium is composed entirely of elongated cylindrical cells.

In the next stage (fig. 9), however, the distal wall of the oldest pulmonary sacculæ has appeared, and is seen still better developed in fig. 8c. The formation takes place as follows: A cleft (*cl. 1*) in the epithelium appears on its internal surface at the junction of the posterior and distal sides of the appendage, while a similar cleft (the first pulmonary furrow, *f. 1*) is formed almost simultaneously on the outer surface. The cylindrical cells between these two clefts immediately begin to shorten to about one-half of their former length and rearrange themselves as a one-layered epithelium, whose basal and free surfaces are now represented by the internal and external clefts respectively.

The proximal surface of the first pulmonary furrow is still

bounded by the original cylindrical cells (figs. 8B and 8c). In these two figures we see, however, the commencement of a second internal cleft (*cl.* 2) and a second external furrow, the latter being the second pulmonary furrow (*f.* 2). In a later stage the cells between the second internal cleft and the two external furrows are seen in the process of shortening to one half of their former length in order to re-arrange themselves to epithelia having this cleft and the two furrows for their basal and free surfaces respectively (figs. 10 and 11).

The walls of the oldest saccule, embracing the first pulmonary furrow between them, and the distant wall of the second saccule, are, therefore, now present. In a similar manner the proximal wall of the second saccule and the walls of all subsequent saccules are formed (fig. 15).

The external pulmonary furrows are always provided with a distinct lumen, cutting deep into the sides of the appendage and sac, and they have been thus figured by Simmons, who was, I believe, the first to observe them. The internal clefts, on the contrary, have no real lumen whatever, and are indicated on the visceral surface of the epithelium by slight grooves only.

The process described above may be summarised as follows : The cells of the epithelial region which contains the pulmonary furrows shorten and re-arrange themselves in the form of a folded epithelium, which has one half the thickness of the original epithelium and occupies about the same total volume.

By repeated cell-division the folded epithelium expands in such a manner that all those folds which are directed inwards and which contain a pulmonary furrow between their walls grow into and ultimately fill out the cavity of the appendage (figs. 10 and 16A). Each hollow pouch thus produced gives rise, of course, ultimately to a hollow air-containing saccule, while the outwardly directed folds comprised between two pulmonary furrows ultimately form the septa between the air-compartments of the lung-book. The

oldest saccule is the first to grow into the interior, while the others follow in turn in the order in which they were formed.

Simmons ('94) is, so far as I am aware, the only author who has described and figured the earlier stages of the formation of the saccules in spiders. He gives two figures of sagittal sections from embryos of the same age, one (fig. 6) showing five and the other (fig. 5) two pulmonary furrows. The position of these two furrows in the latter figure shows that they are not the two oldest, the others having apparently been missed by the section, which is probably of about the same stage as his fig. 6. Simmons' account is as follows: The outer wall of the pocket "has its ectoderm thrown into folds," the nuclei in this ectoderm "being rather irregularly arranged, the pulmonary ingrowths [i. e. the furrows] forcing their way between them." The more distal gill-lamellæ (by which the author means the septa) are the oldest, as in *Limulus* (p. 217). Simmons' paper is dealt with again further on (p. 36).

Comparison with the gill-books of *Limulus*.—It would be profitable here to institute a comparison with the gill-books of *Limulus*.

According to the description and the figures of Kingsley ('85), the gill-leaves of the American *Limulus* arise as outgrowing folds of the epithelium of the posterior side of the appendages, their formation being accompanied by a slight in-tucking of the epithelium between them, and taking place in the same order as the pulmonary saccules in spiders. The epithelial walls of each outwardly directed fold are, however, not in contact along their basal surfaces, and have apparently not been suddenly reduced in thickness, thus differing in these two points from the rudimentary lung-books.

In the Japanese *Limulus* the process appears to be somewhat different. According to Kishinouye ('91), the proximal portion of the appendage is much thicker than the distal portion and is provided with many transverse furrows or invaginations, the tissue between two furrows giving rise to a gill-lamella. At any rate both forms agree in one main

point, namely, that the out-growths or out-foldings are accompanied by an invagination of the ectoderm between them in the earliest stage.

Now, in the case of the rudimentary lung-books in spiders, as summarised on pp. 17-20, it is evident that the pulmonary folds cannot be considered as due to simple out-foldings or in-foldings of an epithelium whose thickness was that of the walls of the folds, as is the case in the American *Limulus* at least. On the contrary, they arise by a peculiar process, which results in the transformation of a very thick but even epithelium into a folded one of one half the thickness, but occupying the same volume, and unaccompanied, therefore, by any out-growth or in-growth at first.

I am of opinion that these two modes of forming a folded epithelium are not fundamentally different, for the one may be readily derived from the other. On the contrary, I believe that the method which obtains in the spider is merely an abbreviation of some such process as occurs in the American *Limulus*, being the most convenient one for rapidly throwing a limited area of a very thick epithelium into folds, for this could not easily be done by ordinary folding, as the breadth of the area in question is only equal to the thickness of the epithelium itself. Which of the two methods was the original depends, of course, on the thickness of the appendicular epithelium in the common ancestor, and is a question of but secondary importance. The Japanese form, according to the description of Kishinouye, appears to bear some resemblance to the spider in the origin of the respiratory lamellæ.

The result of the folding in *Limulus* and the spider are at first practically the same in each case, namely, an undulating folded epithelium, and it is only in the subsequent growth of the folds that a real difference between the two cases becomes apparent. For in each the epithelial cells multiply by division in such a manner that the walls of the folds expand and grow, in the case of the spider, into the interior of the appendage, but outwards and away from the latter in

Limulus. We should have no difficulty in imagining a case in which the cells divided so as to cause the folds to expand simultaneously in both directions, and the result would be a structure intermediate between the gill and the lung-book.

The foregoing paragraphs lead up naturally to the simple and ingenious hypothesis first put forward by Kingsley ('85) to explain the derivation of the lung-books from gill-books (see Kingsley's explanatory figs. 18-20). He simply assumes that simultaneously with the sinking of the whole organ the inwardly directed folds of the gill-books became exaggerated, while those directed outwards correspondingly decreased. In this way an intermediate type of respiratory organ would first be obtained, representing the condition in the animal when it was leaving the water and seeking a terrestrial life. Finally, the lung-book type would be reached by the complete suppression of any tendency of the folds to grow outwards.

Now, from a morphological point of view there should be no difficulty in accepting this hypothesis. The passage from a gill-lamella with three free outer edges to a lung-septum with only one such edge is perfectly simple and easy to imagine. It now really constitutes the only assumption not directly proved ontogenetically which we have to make in deriving the Arachnid lung-book from a Limuline gill-book. For the two remaining conditions necessary for such an origin, namely, the appearance of the oldest septa on the free posterior side of the appendage and the subsequent subsidence of the latter, are observed embryological facts. To return to the first point, the ontogeny, although it does not exactly prove it, furnishes us, nevertheless, with some evidence which tends to show that the folds were originally designed to grow outwards and not inwards. For, so far as I could make out, the two walls of the most distal septum or outwardly directed fold are formed simultaneously, and followed later by the simultaneous appearance of the two walls of a second fold also directed outwards, and so on (see fig. 11). It cannot be denied that each such fold, on its first

appearance, creates the impression that it was designed and is about to grow outwards, and one is perhaps justified in asking why, if the saccules were originally derived from trachea-like invaginations, the two walls of a saccule do not appear simultaneously as we should expect from a fold originally designed to grow inwards? I do not, however, wish to attach too much importance to this point, as it is very difficult to ascertain with certainty, and would not even then constitute a clear proof either way.

Passing to the physiological side of the question, one benefit derived from the sinking of the gill-leaves into the appendage and of the latter into the body would, of course, as Kingsley says, be protection from the increased wear and tear incidental to terrestrial motion. The delicate gill-leaves with the three unattached edges would be very liable to injury when deprived of liquid support, while a lung-septum, having only one unattached edge, is perfectly secure. At first, no doubt, the gill-leaves would be very sensitive to evaporation, and the cavities between their basal portions in the intermediate stage (fig. 19 in Kingsley, '85) may have formed convenient reservoirs for retaining water to moisten the respiratory surfaces during terrestrial excursions.

Various other theories have been suggested by different authors (Milne-Edwards, '72, p. 56; Ray Lankester, '81, '85a and '85b; MacLeod, '82 and '84; Laurie, '92 and '93) to explain how gill-books like those of *Limulus*, may have been converted into lung-books, but none of them correspond exactly to the embryological facts, so I shall not consider them further in this paper.

Later development of the pulmonary saccules.—I resume the description at the 5-6-furrow stage represented in figs. 13-13B and 16-16E. The interior of the appendage has become nearly filled out by the ingrowing saccules, which push before them the intra-appendicular part of the coelom and ultimately occupy its place. They continue to grow till the anterior side of the appendage is reached. The oldest saccules are still the longest, but are exceeded in breadth by

the younger ones (fig. 13B)—so much so, indeed, that in the dorsal region the latter project for at least half their mass into the body cavity, while the oldest saccules are still entirely contained within the appendage (a condition still apparent at the time of hatching, fig. 17).

The plane of each saccule is still an inclined one, slanting upwards anteriorly, owing to the presence of the genital duct in the now ventral (originally medial) portion of the appendage. When, in later stages, the duct has migrated elsewhere, the saccules come to lie horizontally and parallel to the ventral side of the appendage (figs. 17 and 18). A slight twist in the plane of a saccule may always be noticed in the 5-6-furrow stage, by which each becomes distinctly more horizontal in its anterior region (fig. 13B) than at the orifice (fig. 13A). This twist does not seem to be retained throughout all subsequent stages.

From the 5-furrow stage until the period when the cuticula and the lacunæ first appear in the lung-books the latter present various characteristics, best studied in transverse sections, such as fig. 13B. The ventral wall of each of the saccules (*s.* 1, *s.* 2, etc.), is distinctly thicker than the dorsal wall, its cells being more cylindrical and more numerous, its nuclei more oblong and situated nearer the ventral (basal) ends of their cells, which thus come to have more protoplasm at the free (dorsal) ends than do the corresponding (or ventral) ends of the cells of the dorsal wall. The saccules are each provided with a considerable cavity, but between the closely appressed walls of two adjoining saccules no lumen whatever is found.

With the appearance of the chitinous structures and the blood-lacunæ at the end of the reversion a great change takes place in the appearance of the walls of the saccules, the older ones being, as usual, those first affected. There appear between the walls and cells of two adjoining saccules irregular spaces (*lac.*, figs. 17 and 18), which are at first small, but rapidly enlarge and communicate with one another and with the blood-cavities on the medial and lateral sides of the lung-

book, thus forming a passage for the blood and blood-corpuscles (*bl.c.*) from the one side to the other. All mitoses definitely cease in such sacculs, although they are common enough in the previous stages, as well as in younger not yet chitinised sacculs of all subsequent stages. The two adjacent walls do not, however, lose contact with one another, for each cell of a dorsal wall of a saccul (with a few exceptions) remains united with one or two cells of the ventral wall of the adjacent saccul by means of a column of protoplasm, in the formation of which both or all three cells (*w.*, fig. 18) take part. Owing to the excess of nuclei in the ventral wall of the saccul we often find a column provided with two nuclei at its dorsal and one at its ventral end (*y.*, fig. 18), while some of the cells of the ventral wall become simple plaster-cells unattached to a column (*z.*, fig. 18). Similar double nuclei and plaster-cells are rarely found in the dorsal wall of a saccul. This arrangement of the nuclei is retained through all subsequent stages up to the adult form, and was found in the adults of all other spiders examined.¹ I also found it in embryos of *Agelena labyrinthica*, and it is evidently general amongst Dipneumonous spiders.

The nuclei vary greatly in shape. Many are more or less depressed in the plane of the septa, becoming plano-convex or conical, the plane side facing the chitinous cuticula.

The cells of the ventral wall of the oldest saccul (*s.* 1) require special mention. These also form columns, which attach themselves to the body hypodermis, but the cells of the latter do not contribute to these structures. The nuclei of this saccul are often drawn out in a peculiar way into the thinnest part of the ventral columns (fig. 17). Loey, who describes these columns, considers them to be probably of a muscular nature, but there does not seem to me to be any reason for thinking that they are any more muscular than the columns of the septa. Their greater length is simply explained by the fact that each cell has to form a column, at

¹ The plaster-cells were first noticed by Berteaux ('89) in fully developed spider's lungs.

least as long as the two-celled columns of the septa, in order to allow sufficient space for the blood-corpuscles to pass between the ventral saccule and the outer hypodermis.

Two authors, Locy and Jaworowski, deal with the formation of the definite lung-septa from the embryonic epithelia. According to Locy ('86), whose account differs from mine, the nuclei, which are in parallel rows, become plano-convex and arrange themselves in pairs, the convex side of each nucleus in one row being exactly opposite that of an adjacent parallel row (i. e., of an adjacent epithelium). Ultimately the cells of each pair of nuclei, which thus face each other, come in contact and fuse together to form the columns. The cells of such a pair of rows constitute the two walls of a flat, hollow sac, a respiratory lamella (i. e., a septa). Blood has a free access to the lamellæ at their anterior attachments. (Locy's statement that a septa represents a hollow sac is, of course, incorrect. He apparently considers them attached at their anterior ends only.)

Jaworowski's account ('94, pp. 60-61), is more in agreement with mine. According to him the space between the two layers of nuclei of a septum is filled with protoplasm and the lacunæ appear between the cells, and are at first small and roundish, and later on large and elongate. Jaworowski evidently intends to imply that the columns are the remains of the protoplasm left between the lacunæ, and his fig. 12 illustrates this very clearly. Here two, or even three nuclei may be observed at one or both ends of a column at first, but later on this is rarely or never the case, only one nucleus being found at each end of the column (in agreement with Locy).

The chitinous lining of the pulmonary saccules.—Shortly before the appearance of the lacunæ the walls of the saccules appear to collapse, and on the surfaces of contact, where the cavity was situated, two chitinous membranes are secreted. These pass over into one another at their medial, lateral, and anterior edges, so as to form a flattened chitinous saccule within the epithelial saccule, and are further connected by

innumerable tiny chitinous rods, which are firmly soldered to each membrane and distributed over their entire inner surfaces (*s.* 1, figs. 17 and 18). The ante-chamber is also provided with a smooth cuticula (*cu.*, fig. 18), except in the dorsal growing part (*pulm. prol.*).

The walls of the chitinous sacculs are lined on one (the basal) surface with a thin layer of protoplasm, which is, of course, the matrix, and although this layer may become very thin (as, for instance, in *Agelena labyrinthica*), it is always distinctly recognisable at this stage. Locy could not trace the protoplasm on the chitin away from the columns in *Agelena naevia*, while Jaworowski ('94) describes these columns as amoeboid in shape, sending out processes over the surface of the chitin to connect with those of neighbouring cells of the same epithelium.

The moulting of the lung-books.—It is well known that at each moult of the young spider the entire chitinous lining of both the ante-chamber and sacculs is cast off (Menge, '51, p. 22; W. Wagner, '88, p. 315), and that the ventral walls of the latter produce the innumerable free spines on the surface of the cuticula (W. Wagner, '88, p. 314). Various points of interest still remain to be described in connection with the growth at moulting.¹

Already at the time of hatching we find the sacculs preparing for the first post-embryonic moult, although the latter does not take place until nearly a week later. The epithelia of each saccul expand in a medial, as well as in an anterior direction, considerably beyond the corresponding edges of its primitive chitinous lining, while the lateral and posterior edges remain stationary. The enlarged saccul thus created then secretes over its interior surface a new cuticula forming a second chitinous saccul (*s'*, fig. 34), which encloses the one first formed (*s.*) and differs from it in structure. For its ventral membrane bears over that part of its area which is co-extensive with the primitive cuticular saccul (*s.*) nume-

¹ The following remarks on this subject apply equally to *Attus floricola*, *Agelena labyrinthica*, and *Tegenaria atrica*.

rous short cones (*c.*), not attached to the dorsal membrane, while in the newly added medial portion (*s'*.) the rods are fused with both membranes.

Herein lies the explanation of the greater thickness of, and the larger number of cells in, the ventral wall of the saccules in the earlier stages (fig. 13B) described on p. 29; for we may assume that the ventral wall secretes the numerous minute rods as well as the ventral cuticula of the primary chitinous saccules, and that only their dorsal cuticula is contributed by the dorsal wall of the saccules. Being in contact at first the rods of the ventral cuticula are able to fuse with the dorsal cuticula, but at the first moult and all subsequent moults the two cuticulas are separated by the previously formed chitinous saccule except along the newly added medial and anterior portions. The chitinous saccules first formed are cast off at the first moult, but they previously become squeezed very thin and are thus difficult to recognise as such.

At each subsequent moult the saccules are enlarged in the way described for the first moult, and since in the medial and in the anterior portion of the chitinous saccules at any period of life the rods are found soldered to both membranes, I conclude, generally, that this soldered region represents the portion that was added at the previous moult.¹

My account of the primary chitinous saccules differs from that of both Locy and Jaworowski. The first-named author ('86) describes and figures the dorsal chitinous membrane of each saccule as smooth and the ventral membrane as denticular, but not united to the dorsal one in the embryo in *Agelena nævia*. In my sections of the embryos of *Agelena labyrinthica* the two membranes of the primitive saccules are undoubtedly fused together, exactly as in *Attus floricola*. According to Jaworowski's description, in the

¹ The same appears to be the case in many other spiders, although it has hitherto escaped the notice of investigators: so, *Argyroneta*, *Drassodes*, *Lycosa*, *Philodromus*, etc. There is no special reason why the added region should never have free rods, hence the above statement must not be applied too strictly to all spiders.

embryos of *Trochosa singoriensis* both the membranes bear granules (i. e. the teeth), and from his figures it is clear that these membranes are not fused together.

Both these authors' accounts may very easily be reconciled with one another and with mine, if we assume that their figures represent stages in which the preparation for the first post-embryonic moult had already begun. Locy's figures then would represent sections in which the new cuticula of the dorsal wall of the sacculus had separated from the primary chitinous sacculus and so appeared smooth, while the ventral cuticula would still appear denticulate. It may happen in *Attus floricola* that the ventral wall of the secondary chitinous sacculus (s' .) becomes pulled apart from the primary sacculus (s .), which, adhering to the dorsal wall, causes it to appear as if both walls of the sacculus were provided with denticles. This, no doubt, is the explanation of Jaworowski's statement.

The operculum of the lung-books.—It is well known from the observations of Morin ('87), Kishinouye ('90) and others that the outer epithelium of the pulmonary appendage forms the operculum, which covers each lung-book after the appendage has sunk into the body.

It will be observed from a comparison between figs. 13B and 17, and between figs. 16A or 16B and 18, that the sides, as well as the distal wall, of the abdominal appendage contribute to the formation of the operculum. Thus, in fig. 17 the ventral portion, $w'.x'$., of the operculum, to which the ventral columns of the oldest sacculus, $s.l$., are attached, correspond to the ventral wall, $w'.x'$., of the appendage in fig. 13B, while the distal and dorsal walls, $x'.y'$., and $y'.z'$., of the latter correspond as nearly as possible to the portions $x'.y'$., and $y'.z'$., of the operculum in fig. 17 (both figures being magnified the same number of times). A line (*pr. ax.*) through the centre of the area $x'.y'$., or, say roughly, of the entire operculum, and perpendicular to its surface would, I think, correspond approximately with the original axis of the appendage.

Since the positions of the septa and the operculum remain

practically unchanged after the stage represented in fig. 17, we can distinguish in the operculum of the adult spider (1) a nearly horizontal portion to which the ventral saccule is attached, and which belongs to the ventral surface of the abdomen, and (2) a strongly inclined portion on the lower part of the lateral surface of the abdomen. The horizontal part corresponds to the ventral wall of the embryonic appendage in fig. 3 ($w'.x'$ in figs. 13B and 17), or the median wall of an earlier stage (fig. 1), while the inclined portion, which forms much the greater part, is the distal and dorsal wall of the appendage, i. e.—the part $x'.z'$ in figs. 13B and 17, or the distal and lateral wall of an early stage (fig. 1). Anteriorly the operculum curves strongly towards the median line, and this incurved part corresponds, of course, to the anterior wall of the embryonic appendage (fig. 18). All the surfaces pass over gradually into one another and cannot be sharply distinguished.

The lung-books of the young spider.—Not much remains to be added on the subsequent development.

At the time of hatching the lung-book has much the appearance of fig. 18, except that the pulmonary sac (now the ante-chamber) has much thinner walls, lined with chitin internally, and the dorsal saccules are longer. Moreover, that portion of the epithelium of the pulmonary sac immediately adjoining the spiracle now forms a thin-walled, narrow, hollow neck or stalk (pedicel) connecting the ante-chamber proper with the edge of the spiracle.

This pedicel persists throughout all later stages, and its chitinous lining acts both as an air-passage to the ante-chamber and as a sort of ligament by means of which the lung-complex is firmly attached to the outer cuticula of the body.

The dorsal horn of the ante-chamber preserves its characteristic curved form, and, as Bertkau ('72) showed long ago, continues to provide new lung-septa. According to W. Wagner ('88), the addition of new septa goes on until the age of sexual maturity is reached. In *Attus floricola* at the

time of hatching there are about seven or eight developed saccules. At the time of the second moult there are perhaps twelve to fourteen, while in the adult about thirty-four or thirty-five appear to be present, but I cannot state the exact numbers with certainty.

Critical remarks on the literature.—*Araaneæ*.—According to Locy ('86, p. 81) the in-foldings for the lung-books in *Agelena naevia* arise late in the period of the reversion. From his figure (fig. 73) and description of "early stages" (p. 89), in which the lung-books appear as extensive groups of cells with the nuclei arranged in parallel rows, as well as from the fact that he makes no mention of any connection with the abdominal appendages, it is clear that Locy was really dealing with late stages after the appendage had already sunk into the body and long after the earlier saccules had been formed. Of the formation of these latter he gives no account. His account of the formation of the definite septa has already been dealt with on a previous page (p. 31).

Bruce's ('86a, '86b, '87) statements may be dismissed as disproved by later researches. Both Kishinouye ('90) and Simmons ('94) are of opinion that Bruce ('87) has misinterpreted the parts in his figures lxxix and lxxix'. Certainly the fold *L'* is not a pulmonary fold, and is not on the anterior surface of the first abdominal appendage, as Bruce supposes it to be.

Simmons ('94) states that the pulmonary sac arises as an in-pushing behind and under the abdominal appendage, "so that eventually a pit is formed, actually extending into the general body surface." The pit is considered as bounded on its outer side by the appendage itself, its outer wall being described as "the morphologically posterior surface of the appendage" (p. 217), which is represented as lying flat on the body surface and directed backwards. The opening of the pit under the posterior or distal end of the appendage persists as the spiracle. The outer wall of the pit "has its ectoderm thrown into folds, the rudiments of the leaves of the lung-book," and sections of early stages are figured, one

section (fig. 6) showing five pulmonary furrows and the other (fig. 5), although of the same age, only two such furrows.

It is plain that the author considers that the earliest lung-leaves are formed entirely within the pulmonary pit or sac and not on any part of the free surface of the appendage outside of the sac; so that, as far as the position of the lung-leaves in regard to the appendage at their first appearance is concerned, the author has not advanced beyond what was known to his predecessors. Nevertheless, in the summary at the end of the paper we find the following statement, that "the lung-book of the spider (and presumably of all Arachnids which possess one) arises at first as an external structure upon the posterior surface of the abdominal appendages" (p. 219).

If we accept the theory that the lung-books are derived from gill-books as indisputable, then we can say that the appearance of the lung-leaves on the outer or anterior wall of the pulmonary sac proves that this wall is morphologically the posterior side of the abdominal appendage, but we cannot conversely first call this wall the posterior side of the appendage and then say that the appearance of the lung-leaves upon it proves that they are formed on the posterior side of the appendage, as Simmons does. For if we choose to consider that the lung-books were derived from internal tracheæ and not from external gill-books, the pulmonary sac would be the trunk of a trachea, and no one would then call its outer wall the posterior wall of the appendage. Thus, if Simmons' description of the early development were correct, then the lung-books would not arise at first as an external structure, but as an internal one in an invagination.

As a matter of fact Simmons' representations of the abdominal appendage in his figs. 5 and 6 are very misleading, as will appear if we refer to his fig. 10, which represents an entire embryo of the same age as those in figs. 5 and 6. Here the first abdominal appendage has its usual stumpy, knob-like form, and is situated on opposite sides of the abdomen, almost antipodal in fact, just as in *Attus floricola*.

Sagittal sections, like Simmons' figs. 5 and 6, therefore, cut the appendage more or less transversely to its main axis, which in the two figures would be, not in the plane of the paper, but almost perpendicular to it.

In fact I cannot believe that the appearance of the appendage in *Agelena naevia* at this stage differs so essentially from the corresponding stage in *Attus floricola*, such as that represented by my fig. 16. A sagittal section in the case of the appendage represented in this figure would, of course, be more or less perpendicular to the plane of the paper and cut the appendage parallel to the line *ep.-ep.* If the section were slightly more inclined towards the lower part of the paper (say along *a.-b.*, fig. 16B) we should get a section like fig. 15, but if it were inclined more towards the upper part of the paper (say along *c.-d.*, fig. 16B), we should get sections exactly resembling Simmons' figs. 5 and 6, according as two or five of the furrows were cut. This I believe to be the true explanation of the appearance of Simmons' figures. It is extremely difficult, if not impossible, to get an exact idea of the structure of an appendage without the aid of wax models, of which Simmons does not say he made any use.

The last paper on the spider's lung-book to be considered is that of Jaworowski ('94), who studied *Trochosa singoriensis*. He discovered in this species an embryonic structure, which he describes as an embryonic trachea, consisting of an ante-chamber, a trunk, and branches. The ante-chamber is inverted funnel-shaped, with the apex pointing upwards and the broad end terminated ventrally by the abdominal appendage or operculum. The sides of the ante-chamber are closely appressed to one another (p. 56) and extended in a sagittal plane (since they are seen broadways in sagittal sections). The pulmonary lamellæ are formed by parallel folds of the wall of the ante-chamber, "the edges of the folds, which jut into the lumen, being more or less (figs. 1 and 2) undulate" (p. 62) and parallel to the surface of the operculum, i. e., transverse to the axis of the ante-chamber and trachea.

According to Jaworowski's idea, therefore, the free edges

of the septa run parallel to the longitudinal axis of the abdomen instead of at right angles to it, as they do in *Attus floricola*, etc. Now if we compare my figs. 13B and 17 of transverse sections with Jaworowski's figs. 3 and 5,¹ which come just in between mine in point of development, it will be seen that the lung-books of both species exactly correspond, so that the free edges of the septa cannot run longitudinally to the body axis. In fact, Jaworowski has evidently mistaken the direction of the folds, which are seen laterally in his figs. 3 and 5 and not from their free edges; and, moreover, the funnel-shaped area which he calls the ante-chamber in his sagittal sections is not the ante-chamber at all.

The trunk of the embryonic trachea, according to Jaworowski, extends dorsad from the apex of the ante-chamber and then divides, the branches reaching to the dorsal blood-vessel and subdividing into smaller branchlets. These have sometimes the appearance of a cuticular tube provided with regular internal thickenings (fig. 6). Ultimately both trunk and branches degenerate and disappear, only the "ante-chamber" remaining to form the lung-book.

In the later stages of the spider-embryos which I examined, I find the yolk-mass continuous along the median region but divided towards the sides by partial septa, which are transverse and doubtless of mesodermal origin. The surfaces of the yolk are lined with very thin flat cells, and the intra-septal space between these two layers of cells contains muscles, blood-corpuscles, and a number of large vitellophagous cells resembling those marked *vit.* in my figs. 16D, 16E, 17, etc. Ventrally the intra-septal spaces widen out, the widened part appearing funnel-like in sagittal sections (see fig. 41, which shows three such septa). The lung-books lie in the ventral widening of the septum between the eighth and ninth segments. The space between the lung-books and the

¹ The author calls these "frontal sections," but since the abdomen is inclined ventrally to the longitudinal axis of the cephalothorax, frontal sections of the latter would cut the abdomen more transversely than frontally. (See Locy's fig. 10 or Korschelt and Heider, p. 585, fig. 372B.)

yolk also contains blood-corpuscles, vitellophagous cells, and various mesodermal elements, besides fluid.

Jaworowski's tracheal trunk and ante-chamber undoubtedly correspond in position to the lower part of the septum and its funnel-shaped widening, but I have found nothing in them in my sections which could possibly be taken for tracheæ. Jaworowski states that the trunk has a nucleated epithelium, the nuclei being smaller than those of the pulmonary lamellæ (p. 62). These may well be, I think, the nuclei of the mesodermal septa, but I am at a loss to account for the tracheal branches and branchlets drawn by Jaworowski in his figs. 1 and 2. At any rate the tracheal nature of the structure cannot possibly be maintained so long as no embryological evidence at all is advanced to prove that they are of ectodermal origin and derived from the same mass of cells which form the lung-books. It will be noticed further that the lumen of the ante-chamber is closed off from that of the tracheal trunk by a diaphragm (p. 63).

No other investigator has ever found anything like these embryonic tracheæ, and although Jaworowski ('94, p. 55) asserted that Schimkewitsch ('86a, '86b) had previously observed a similar structure, the latter author has recently (:06, p. 45) disclaimed any connection between that figured by him and those found by Jaworowski.

Scorpiones.—Metschnikoff ('71), Laurie ('90 and '92), Brauer ('95) and Pereyaslawzewa (:07) all agree that the lung-books of scorpions arise as folds in the wall of the pulmonary sac, which according to the first three authors is formed by invagination on the posterior sides of the four posterior pairs of abdominal appendages. According to Pereyaslawzewa, however, this sac arises on the anterior side of the appendages, but it appears to me probable that this author has mistaken the intersegmental folds which separate the sternites for appendages, the true appendages described by previous authors having evidently already disappeared.

Brauer states that, so far as he could make out, the oldest pulmonary fold occurs at the innermost part of the sac, the

following folds occurring on the distal side of this one (i. e. exactly opposite to what takes place in spiders). The author does not appear to be quite certain about this point, and is, moreover, corrected by Pereyaslawzewa, who maintains that the oldest fold is the one nearest to the outer body wall (i. e. as in spiders).

Brauer's text-fig. 15c (p. 413) very closely resembles my fig. 16b, so far as the ectoderm is concerned. He thinks there can be scarcely a doubt that the lung-book is not formed behind or apart from the appendage, but is the posterior half of the latter itself, which is invaginated and on which the pulmonary folds appear (p. 415).

Laurie ('92) makes an interesting statement regarding the position of the lung-septa in the older scorpion-embryos, Here they are placed horizontally, as in the older spider-embryos, whereas in the adult scorpion they are vertical (p. 102).

Pedipalpi.—The development of the lung-sacculæ and their relation to the abdominal appendages do not appear to me sufficiently clear, from the existing embryological data, to make a comparison with the Araneæ possible. Apparently the abdominal appendages are not so obvious in this group as they are in Araneæ and Scorpiones, since the parts described by Laurie ('94) under this name are not identical with those to which Schinkewitsch (:06) applies the term.

A remarkable point in the development, as described by Schinkewitsch, is that the oldest sacculæ are said to be formed within the pulmonary sac and to subsequently migrate out of it on to the posterior side of the appendage. In such a case their development would be exactly the opposite to that in Araneæ, as well as to what we should expect from phylogenetic considerations.

Pereyaslawzewa's (:01) description of the formation of the lung-septa out of the cuticular wrinkles of the body-surface is altogether fanciful.

The fully-developed lung-books of spiders.—A. Schneider ('92) has given an excellent account of the coarser anatomy of the

lung-books in spiders, the descriptions of MacLeod ('84) and Berteaux ('89) being unsatisfactory in this respect. Berteaux's account of the chitinous structures (spines, etc.) of the lung-books is, however, very detailed and the best we possess, but his description of the bi-nucleated cell-columns in the septa as unicellular structures is misleading and not in accordance with the embryological facts, since these columns are formed by the fusion of opposed cells in two separate epithelia.

Both MacLeod and Berteaux made a curious error regarding the free edges of the septa. The edges of the septa they describe as being free, not only along the posterior border but along the posterior part of the lateral side as well. As a matter of fact the lateral sides of the septa are never free, but may have the appearance of being so in horizontal sections through the dorsal procurved portion of the antechamber. The apparently free edge is merely that of a septum with its lateral part cut off by the razor, hence the irregularities in its occurrence observed by these authors. That these lateral edges are not free can easily be demonstrated by examining the lung-books under a hand-lens after treatment with caustic potash, and I can strongly recommend this old-fashioned method to anyone who wishes to obtain a clear idea of the coarser structure of the lung-books in a short time (see fig. 20). It will be found much more satisfactory than if one were to rely on sections only.

Börner (:04) has recently stated that the septa are placed more or less vertically in the majority of the Araneæ, and has thrown doubt on MacLeod's well-known diagrams, in which the septa are represented as lying horizontally.

I have examined one or two species of most of the larger families and I found the septa as nearly horizontal as they could well be in the following Dipneumonous spiders: Attidæ (*Attus floricola*), Lycosidæ (*Lycosa Darlingtoni*), Agelenidæ (*Tegenaria domestica*, *Textrix lycosina*), Clubionidæ (*Clubiona holosericea*, *Palystes* sp.), Thomisidæ (*Philodromus fuscomarginatus*), Theridiidæ (*Theridion lineatum*), Drassidæ

(*Drassodes tessellatus*), Sicariidæ (*Scytodes tessudo*); also in the following Tetrapneumonous spiders (Aviculariidæ): subfam. Aviculariinae (*Harpactira atra*), subfam. Ctenizinae (*Stasimopus unispinosus* and *Hermacha* sp.).

In the following forms the septa were inclined at an angle of 45° or less to the horizontal, sloping downwards from the higher medial edges to the lower lateral edges: Argiopidæ, subfam. Argiopinae (*Argiope clathrata*), Theridiidæ (*Latrodectus geometricus*), and Eresidæ (*Eresus* sp.).

If the above examples are any indication of the usual position in the families to which they belong, then Börner's statement must be wrong, and cannot hold good for the great majority of Araneæ. Even in the three cases where the septa were inclined they were nearer the horizontal than the vertical (except, perhaps, in *Latrodectus geometricus*, where they formed an angle of about 45°).

Moreover, the operculum in the spiders with horizontal septa is similar to that of *Attus floricola* described on p. 49 (see fig. 17), and since this type of operculum represents that of any Dipneumonous or Tetrapneumonous spider in which the abdomen is not greatly developed anteriorly, we may fairly assume that the septa must be horizontal, or very nearly so in the great majority of spiders.

When, however, the anterior upper region of the abdomen is abnormally distended above the opercula, it may happen that the lateral region of the latter becomes pushed downwards into a more horizontal position than is the case in fig. 17, and at the same time the septa become tilted upwards on the medial side, that is to say, they take a more or less inclined position, such as one finds in *Argiope*, *Latrodectus*, and *Eresus*, and no doubt in many other genera of the same families. The inclined position of the septa cannot, therefore, be a primitive condition in these families, but, I think, merely due to the abnormal distension of the abdomen, for in closely allied forms, in which the abdomen is not

unusually distended and the operculum more upright, I find the septa placed almost horizontally (e. g. in a specimen of *Nephila* from Senegal).

VI. THE DEVELOPMENT OF THE ABDOMINAL LONGITUDINAL MUSCLES AND THEIR TENDONS.

In their earliest stage the cœlomic sacs of the eighth to eleventh segments each protrude an evaginated portion of their somatic wall into a provisional appendage, completely lining the cavity of the latter (fig. 4). At this stage (*St.* 1) the cells of the somatic wall of the sac are cylindrical, and much higher than those of the splanchnic wall.

At the time when the first pulmonary furrows begin to appear (stage 2) the intra-appendicular portion becomes partially cut off from the main cœlomic sac by an infolding of its wall along the medial basal edge of the appendage (fig. 1). The infolded layer grows in a lateral direction halfway across the cavity of the appendage, converting the medial half of the intra-appendicular cœlom into a short tube. Each of these segmental tubes lies in a transverse plane, is blind at the medial end, and opens laterally into the cœlomic sac.¹

In a longitudinal section through the lateral region of the appendages (*ab. app.* 1-3, fig. 5A) the main cœlomic cavity is seen to be continuous with the intra-appendicular portion, whereas the latter portion in a more medial section of the same series (fig. 5) appears separated from the main cœlom, the segmental tubes (*seg. t.* 8-10), of course, being seen in cross section. If this latter section be compared with a corresponding section of a later stage (fig. 6, stage with five pulmonary furrows), it will be noticed that the portion of the somatopleura which formed the inner layer of the above-

¹ I have shown elsewhere ('95) that the segmental tubes of the eighth or pulmonary segment become the genital ducts. In fig. 23B (adult male) the mesodermal part of the genital duct (*mes. g. d.*), derived from the segmental tubes, is seen sharply differentiated from the ectodermal portion (*ec. g. d.*).

mentioned fold, and which overlies the segmental tube, has increased considerably in thickness in each segment. Its cells (*m.*, fig. 6) are no longer cubical and one-layered, as they are in fig. 5, but have become spindle-shaped, elongated longitudinally to the body, and arranged in several layers. Each such group of elongated cells forms a bundle whose ends are in contact with the anterior and posterior basal edges of the appendages, thus completely bridging over the segmental tubes, and ultimately gives rise to a corresponding segment of one of the two great ventral longitudinal muscles of the abdomen.

The ectodermal areas to which these muscular segments are attached are of primary importance in enabling us to determine the homologies of the tracheæ. In their earlier stages it will be seen that these areas (*ar.* 7-11, figs. 6 and 27) are precisely similar to each other in position and arrangement with regard to each appendage. They occupy the visceral surface of the medial half of each post-appendicular (intersegmental) in-folding,¹ and their transverse extension is at first nearly the same as that of the segmental tubes with which the areas alternate.

Each contact area soon becomes marked by the appearance of the intermuscular tendons or entochondrites. These organs, whose mesodermal origin has already been demonstrated by Schimkewitsch ('94) are formed in various parts of the body by the fusion and metamorphosis of the ends of the muscular cells at those places where the ends of two or more muscle-bundles come in contact with each other and with the hypodermis.² In the embryo they form a non-

¹ For the area (*ar.* 7) between segments 7 and 8 there is, of course, no post-appendicular in-folding, but its position is otherwise precisely similar to that of the others.

² Schimkewitsch, to whom we mainly owe our knowledge of the development of the entochondrites in Arachnida, has a somewhat different view of the conditions necessary for the formation of these organs. He says ('94, p. 206): "Mann kann behaupten, dass in jenen Fällen, wenn zwei Muskelanlagen einander entgegen wachsen, sie mit einander verwachsen; wenn sie aber unter einem Winkel zusammen-

nucleated homogeneous mass, which stains very deeply with carmine, and is, histologically, readily distinguishable from all adjacent tissues. All the cells of the ventral longitudinal muscles become subsequently attached by each end to these entochondrites only, which in turn firmly adhere to the bases of the ectodermal cells of the deepest part of the contact-area described above, and provide us with a certain means of locating these latter cells long after the subsidence of the appendages. It should be noted that the entochondrites are much less extensive at first than the ectodermal areas (*ar.* 7, etc., figs. 6 and 27) with which the ends of the forming muscles were originally in contact.

From what has just been said, there can be no doubt that the places of attachment of the entochondrites inserted in each ventral longitudinal muscle are serially homologous, a fact which may also be readily inferred from a glance at fig. 41, which represents the stage shortly before hatching. From this figure it may also be observed that several other muscles are attached to the entochondrites (*t.* 7-11), in addition to the segments (*v. l. m.* 7-11) of the great longitudinal muscles as, for instance, (1) a long dorso-ventral muscle (*d. v. m.* 7-10); (2) a short, oblique muscle (*p. ob. m.* 8-11) running obliquely forwards and attached near the middle of its segment to the hypodermis without the interposition of an entochondrite; and (3) a corresponding oblique muscle¹ (*a. ob. m.* 8 and 10), running obliquely backwards, present in segments 8 and 10. In *Agelena labyrinthica* I also

treffen, so bildet sich an der Stelle ihrer Berührung eine Sehne mesodermalen Ursprungs." To my mind it does not matter whether the muscles are in a line or meet at an angle, the essential conditions being only that the ends of two or more muscles should meet at one spot and that that spot should be on the basal surface of the hypodermis. When muscles are attached singly to the hypodermis they do so directly without the intervention of an entochondrite.

¹ I have called this muscle-series and that previously mentioned the anterior (*a. ob. m.*) and posterior oblique muscles (*p. ob. m.*), because they lie in the anterior and posterior regions respectively of their somites.

found an oblique muscle on the medial side of the entapophysis of the pulmonary segment, which, perhaps, corresponds to a muscle (*a. ob. m. 9*) not observed in *Attus floricola*.

In the embryo and the growing spider the ectodermal areas to which the entochondrites of the longitudinal muscles are attached become drawn out in the form of hollow processes (*ec. t. 8-11*, fig. 41), lined with chitin, and projecting into the interior of the body. These are the ectodermal tendons or entapophyses¹ (apodemes) of the ventral longitudinal muscles, and it was the purpose of the foregoing paragraphs to establish their serial homology.

Since the segments of the longitudinal muscles are each formed out of the entire length of the somatic wall of a cœlomic sac, it follows that their points of attachment (or the entapophyses) must be intersegmental, and this is also the case with the points of attachment of the dorso-ventral muscles where they coincide with those of the longitudinal muscles (as, for instance, the ventral end of the muscle *d. v. m. 8*, and both ends of *d. v. m. 9* and *10* in fig. 41). But it frequently happens in the Arachnida that the dorso-ventral muscles disassociate themselves at one or both ends from the insertions of the longitudinal muscles, and may then form separate external depressions, which are not necessarily intersegmental, but, in fact, very frequently placed more forwards on the face of the segment. Thus in fig. 41 the muscle *d. v. m. 8*, although actually in contact at the place of crossing with the dorsal longitudinal muscle (*d. l. m. 8*) of the eighth somite, is inserted dorsally at some distance in front of the intersegmental entochondrite which lies between the muscles *d. l. m. 8* and *9*.

VII. THE ENTAPOPHYSES (ECTODERMAL TENDONS) OF THE PULMONARY SEGMENT.

When the entochondrites begin to form at about the stage with five pulmonary furrows, the first pair of provisional appen-

¹ A term introduced by Ray Lankester.

dages has reached its most lateral position (fig. 3). The anterior wall of appendage 2 has approached nearer to appendage 1, so that the ectodermal area (*ar.* 8, figs. 6 and 16), to which the entochondrite becomes attached, now forms the bottom of a rather wide groove lying between the two appendages towards their medial side. Shortly afterwards the three posterior provisional appendages commence to move towards the posterior ventral part of the abdomen away from appendage 1.

Now, as the latter retains its lateral position for the present and is very close to appendage 2, we find the area (*ar.* 8) between them, to which the tendon is attached, also shifting slightly ventrad. In fig. 27 the appendages are still in a row, but the ventrad movement is commencing; in fig. 16 (a slightly later stage) appendage 2 has moved ventrad to the region comprised between the sections nos. 1-18, and the area of attachment (*ar.* 8) is no longer just behind the region of the two oldest furrows (*f.* 1 and *f.* 2), as it appears to be in fig. 27, but lies ventrally to them. When this area has reached the extreme postero-ventral corner of the base of the appendage, it remains there, while appendage 2 continues its ventrad movement.

The subsequent development up till after the second moult is a simple one. After the completion of the reversion and shortly before hatching (stage 6) we find the entochondrite (*t.* 8, fig. 41) situated alongside the posterior median corner of the lung-complex (*lb.*), just next to the medial end of the spiracle. The ectodermal area (*ec. t.* 8) to which the entochondrite is attached, is composed of elongated cells and is somewhat sunken-in, forming a shallow, groove-like continuation of the spiracle (*sp.*, fig. 18). At the second moult, when the young spider attains its definite form, this groove becomes obliterated, so that the hypodermis of the attachment area comes to lie on a level with the adjacent body surface and completely outside of the spiracle.¹

¹ In *Agelena labyrinthica* the groove is present in all stages after the time of hatching.

As the young spider, however, approaches maturity, the attachment area again undergoes a considerable alteration in its form and position, resulting mainly from two processes. These consist in (1) the formation of a transverse in-folding of the hypodermis (the interpulmonary fold) between the two spiracles, which thus become connected by a deep ventral groove; and (2) the drawing-out in the form of a blind tube (entapophysis) of the dorsal edge of this fold at the two spots to which the pair of mesodermal entochondrites are attached.

The interpulmonary (epigastric) fold in the adult of *Attus*.—Fig. 20 represents a posterior view of the abdominal chitinous skeleton of an adult of a species of *Attus*, after the removal of everything posterior to the transverse plane which passes through the spiracles. It will serve to illustrate the form of the interpulmonary fold in the adult.

In *Attus floricola* the interpulmonary fold in the male differs greatly from that of the female.

Figs. 23-23B are three sagittal sections through this fold in the adult male in the regions indicated in fig. 20. The much crumpled, anterior and posterior surfaces of the groove contained in the fold are normally closely applied to each other, so as to leave very little space between them. Along the dorsal edge, however, this is not the case, for here the groove suddenly widens to a nearly cylindrical canal (*can.*), which opens on each side into the pulmonary ante-chambers at their medio-ventral corners, thus forming a permanently open communication between the two lungs ["canal of communication" observed by Berteaux ('89) in *Agelena* and *Epeira*]. The chitinous wall of the canal is thick and covered with branched anastomosing spines, quite similar to and directly continuous with those of the ante-chambers.

The two entapophyses (*ec. t.* 8) have each the form of a short, strongly compressed pouch, whose blind end is directed upwards, backwards and laterad. The cavity in the ventral part of each branches off from the interpulmonary canal, and is provided, like the latter, with anastomosing

spines (*spi.*, fig. 23A), while near its blind dorsal end it is without any spines (*cc. t. 8*, fig. 23A).

The hypodermis of the blind end is of special interest. Its cells assume a fibrous structure (*hy'*, fig. 23) and are not pigmented like the adjacent hypodermis, and the large entochondrite (*t. 8*) is firmly attached to their basal ends.

The numerous and powerful muscles which are attached to this entochondrite have been described by Schimkewitsch ('84), Vogt ('89), A. Schneider ('92), and others. It corresponds to the anterior of the three pairs of abdominal entochondrites described by these authors.

In females of *Attus floricola*, at any rate in matured or nearly matured specimens, the interpulmonary fold differs in a very remarkable manner from that just described. Instead of the cylindrical spinous canal of communication one finds a broad, thin-walled, much wrinkled, band-shaped canal, without spines internally, and strongly compressed from before and behind (*can.*, fig. 22). Moreover, the two walls of the fold itself, apart from the canal, are much more strongly wrinkled than is the case in the male.

In the adults of both sexes the opening of the genital organs is found on the anterior wall of the medial region of the fold between the pair of entapophyses (*g. o.*, figs. 20 and 23E).

The interpulmonary fold in other spiders.—The interpulmonary fold¹ was found in all Dipneumonous spiders examined by me, and in the majority of the genera resembled the conditions occurring in either the male or the female of *Attus floricola* (*Marpissa* ♂ ♀, *Clubiona* ♀, *Agelena* ♀, *Pisaura* ♀, *Dolomedes* ♀, *Melanophora* ♂ ♀, *Drassodes* ♀, *Zora* ♀, *Linyphia* ♀, etc.).

The pair of entapophyses are very variable in shape, even in the same species; thus out of five specimens of *Tegenaria domestica* (fig. 21) examined, no two had the entapophyses shaped exactly alike. In some forms these tendons may be

¹ This fold was known to Treviranus ('12), and is described by MacLeod ('84), Berteaux ('89) and others.

far apart (*Attus* sp., fig. 20), in others, again, close together (*Tegenaria domestica*, fig. 21), or even fused to a single, rounded, median lobe (*Lycosa Darlingi*). The free end or ends are either sub-entire or else drawn out into short finger-like processes (fig. 21). Except in the *Dysderidæ* the entapophyses are more or less inclined backwards.

The interpulmonary canal varies considerably, and is frequently spined and even cylindrical in the female as well. In the majority of cases it forms a canal of direct communication between the ante-chambers of the pair of lung-books, as in *Attus floricola*, but in the *Lycosidæ* and in *Philodromus* this is not the case, the interpulmonary fold being rudimentary in the lateral part between the entapophysis and the lung-book. In many *Lycosidæ* this portion of the fold is represented merely by a slight internal thickening of the cuticula (*interp. fld.*, fig. 19A), but in *Philodromus* there is a slight in-folding of the outer surface as well (fig. 19B) without an actual lumen being formed. The presence of these rudiments indicates that there was once a well-developed fold connecting the pulmonary sacs with the entapophyses, and that the present condition is a secondary one.

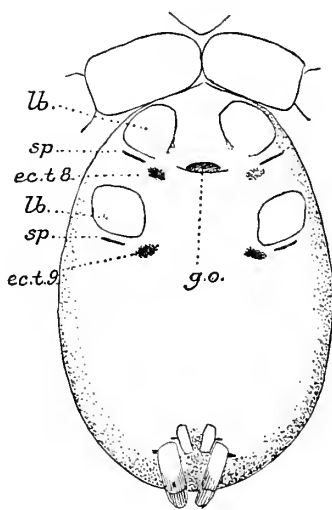
In *Argyroneta* (♂) the fold is well developed throughout, but there is no canal of communication, the two surfaces of the fold being closely apposed and without a lumen between them (fig. 19c).

In the *Dysderidæ*, too, there is no spinous canal of communication, although in the median part the lumen may be widened (*interp. fld.*, fig. 40). In *Dysdera* and *Segestria* the fold is deep and well developed between the pair of entapophyses, but on the lateral side of these it is rudimentary and not continuous with the spiracle. In *Harpactes* the fold is much less deep than in the two other genera, and the entapophyses are hardly specially distinguishable at all, being merely slightly deeper portions of the fold, to which the entochondrites are attached. The lateral portion of the fold is, how-

ever, here directly continuous with the spiracles. The male (but not the female) of *Harpactes* is also remarkable in that the opening of the genital organs lies immediately in front of, but separate from, the interpulmonary fold (*g. o.*, fig. 40), whereas in all other Dipneumonous spiders examined the genital opening lies in the anterior wall of the fold.

In the Tetrapneumonous spider¹ examined I found no inter-

TEXT-FIG. 2.



2.

Crypsidromus intermedius. Ventral surface of abdomen.

lb., opercula of lung-books; *sp.*, spiracles of lung-books;
ec. t. 8 and *9.*, muscle insertions; *g. o.*, genital opening.
 Magnified 6.

pulmonary fold connecting the spiracles, but on the medial side of the latter (but separate from them and from the genital cleft) shallow depressions resembling stigmata in the cuticula were observed (*ec. t. 8* and *9*, text-fig. 2), which proved in sections to be the places to which the entochondrites of the ventral longitudinal muscles are attached. These rudimentary

¹ Specimens labelled "*Crypsidromus intermedius*, Paraguay," obtained from the Berlin Zoological Laboratory.

entapophyses (*ec. t.* 8, fig. 36) were similar in both pulmonary segments.

The only other order possessing the interpulmonary folds is the Pedipalpi, in which these folds are very well developed in both pulmonary segments and much resembles that of Dipneumonous spiders (see Tarnani, '89 and :04, and Börner, :04).

VIII. THE DEVELOPMENT OF THE TRACHEÆ AND THE ENTAPOPHYSES OF THE TRACHEAL SEGMENT.

The tracheal appendages are, as nearly as possible, the exact counterparts of those of the pulmonary segment in the earliest stages, up to, say, the period when the pulmonary furrows begin to appear (compare *ab. app.* 1 and 2 in fig. 4). The post-appendicular groove (*gr.*) extends along the whole posterior side of the appendage (except, perhaps, as in appendage 1, at the extreme lateral part), but it does not appear to be deeper laterally than medially.

In the stage with two pulmonary furrows (figs. 1, 5 and 5A), however, after the simultaneous subsidence of the epithelium lying between consecutive abdominal appendages we find that the post-appendicular groove is not almost obliterated in its medial half (*tr. s.*, fig. 5), differing in this respect from the corresponding groove of the pulmonary segment (*gr.*, fig. 5). On the contrary the infolding containing the groove has increased in depth along its whole extent, and continues to deepen in the following stages in such a way that its blind bottom is directed slantingly forwards (*tr. s.*, fig. 6A). This in-folding is the tracheal sac.

If we examine a reconstruction of the appendage from the inner surface (fig. 27) at this stage (when about five pulmonary furrows are present and the mesodermal entochondrites begin to be formed), we find a broad transverse ridge (*tr. s.*) projecting into the body and nearly co-extensive with the posterior side of the base of the appendage. This ridge is the ectodermal in-folding which forms the tracheal sac. The

space (*ar.* 9) occupying the medial region of its visceral surface and enclosed by the dotted lines in the figure is the area with which the ends of the longitudinal muscles are in contact, and to the deepest part of which the entochondrite becomes attached. The medial area (*ar.* 9) of the tracheal sac is, therefore, serially homologous with the corresponding area (*ar.* 8) behind the pulmonary appendage (see p. 20), and has consequently nothing to do with the region in which the earlier pulmonary furrows appear, nor with any portion of the lung-books. It will be observed that owing to the presence of the lung-leaves the area (*ar.* 8) in the pulmonary segment is more widely separated from the segmental tube (*seg. t.* 8) than is the case in the tracheal segment.

The lateral region of the tracheal in-folding is of especial interest, as it is the only part which is serially homologous with the pulmonary sac. It will be remembered that the pulmonary sac proliferates in a lateral direction (position as in fig. 1), later in a dorsal direction (position as in fig. 3), in the form of a hollow tuber-like process creeping along the inner surface of the outer epithelium; and that this sac and its proliferations yield the cell-material for the formation of the fourth and following pulmonary saccules.

Now the tracheal post-appendicular in-folding begins to proliferate laterally simultaneously with the pulmonary sac in precisely the same manner and direction. But the walls of the tracheal sac have not to furnish cell material for lung-saccules, of which no traces are present at any time, and, no doubt, on this account the pulmonary sac rapidly outgrows the corresponding tracheal sac, and in the stage of fig. 27 already greatly exceeds it in size. In this figure the groove (*tr. l.*) behind the tracheal appendage extends dorsally up to section No. 16, while the proliferation extends through five more sections; and the groove (*pulm. l.*) behind the pulmonary appendage reaches to section No. 24, while the corresponding proliferation extends further likewise through five more sections.

Figs. 35-35A and 16D-16E represent longitudinal sections

through the tracheal and pulmonary proliferations respectively of one and the same embryo from a series of sections similar to those from which fig. 27 has been reconstructed and representing the same stage. It is to be noticed that the tracheal proliferation (*tr. prol.*) is solid throughout, while that of the pulmonary sac (*pulm. s.*) is provided with a considerable cavity, though this is, of course, not a fundamental difference but is to be considered rather as due to a mere difference in the rapidity of growth. In other respects both proliferations closely resemble each other: in each the incision between it and the outer epithelium is deepest on the anterior side and dorsally at the apex. The opening of the tracheal sac does not extend dorsally beyond section No. 16 (fig. 6A) of fig. 27, the following five sections (compare figs. 35 and 35A) showing no trace of a post-appendicular groove, exactly as in the case of the corresponding five sections (Nos. 25-29) of the pulmonary appendage. In both cases the dorsal ends of the openings represent the latero-dorsal ends of the permanent spiracles, the medial ends of which are still unformed.

Shortly after the stage I have just described the migration of the three posterior pairs of abdominal appendages, already alluded to on a previous page, commences. This process, which may be considered as characteristic of all Dipneumonous spiders with the tracheal spiracle near the hind end of the body, consists of a double movement, namely, a medio-ventrad movement of each of the three pairs of appendages and a caudad one caused by the enormous elongation of the ninth somite. Near the end of the reversion, as a result of this process, these appendages come together in pairs in the median line in the posterior half of the abdomen (*tr. pl.*, figs. 41 and 43). At the same time the tracheal appendages gradually sink to the level of the body surface.

During this period the formation of the tracheal spiracles is completed, the lateral ends of the spiracles having already been formed at an earlier stage. The unformed median ends become approximated by the migration of the appendages towards the median line, and subsequently the region of the

body-epithelium lying in between (*inf.*, fig. 28) folds into the body, and the two spiracles become united to a single one (*sp.*, fig. 28).

Meanwhile, important changes have taken place in the post-appendicular sac of the tracheal appendage. Fig. 28 represents a reconstruction seen from above of the ectoblast of the two tracheal sacs (together with four muscles and two entochondrites) at the end of the reversion. Fig. 41 is a sagittal, and fig. 43 a transverse section of the same stage. The right half of fig. 28 is equivalent to the tracheal in-folding (*tr. s.*) in fig. 27, dorsal in the latter figure corresponding, of course, to lateral in fig. 28.

We observe in the first place (fig. 41) the great longitudinal elongation of segment 9, bringing the tracheal spiracle (*sp.*) and sac nearer to the posterior end of the abdomen. As if to compensate for this backward migration the medial region of each tracheal in-folding, that is, the region corresponding to the area *ar. 9*, fig. 27, to which the entochondrite is attached, becomes drawn out in the form of an elongated plate (entapophysis, *ec. t. 9*, figs. 41 and 28), which is directed forwards and slantingly upwards and is much compressed dorso-ventrally.

The entochondrite (*t. 9*) is attached to the narrowed anterior end of the plate, and at the extreme posterior lateral corner of the latter a second entochondrite (*t.*, fig. 28) is found attached to those cells which bound the lateral angles of the spiracle. Between these two entochondrites the anterior oblique muscle (*a. ob. m. 10*) is stretched.

The bulging lateral portion (*tr. prol.*) of each plate corresponds to the dorsal proliferation of a previous stage (*tr. prol.*, fig. 27). In the line of the transverse section, fig. 43 (see fig. 28), the lateral edge of the plate (*tr. pl.*) is some little distance from the outer hypodermis (*hy.*) but more posteriorly, near the entochondrite *t.*, fig. 28, the edge of the plate comes nearly or actually into contact with the outer hypodermis.

The pair of plates are connected like the spiracle by the

in-folding (*inf.*, fig. 28) of the epithelium between them, and, therefore, possess a common lumen, which, however, is confined to the basal region only and denoted in the figures by the area, *tr. l.*, within the dotted lines. The greater part of each plate is, therefore, solid. Fig. 28 gives the correct outline of the pair of plates, as they appear near the end of the embryonic period, and I have tested the accuracy of the reconstruction by comparisons with sections cut parallel to the tracheal plates, so as to contain the whole width of the pair of plates in one section. I could not detect a distinct chitinous lining within the lumen of the plate at this stage, for the cuticula which previously covered the body surface always appeared quite loose and outside of the cavity, as if the embryo were undergoing a moult such as Locy describes for *Agelena*.

If the tracheal and pulmonary appendages be now compared, the difference in the relative development of the two main organs connected with them becomes apparent. The entapophysis is small in the pulmonary segment but large in the tracheal segment, where it forms the greater part of the tracheal plate, while the large mass of cells composing the lung-book is represented by the comparatively small, lateral, bulging portion of the tracheal plate.

The post-embryonic development of the tracheal plate.—After the hatching of the embryo very important changes take place in the shape of the tracheal plates. In the first place the medial tendinal portion of each becomes drawn out in a forward and upward direction to form an elongate, spatulate, hollow process, which is strongly flattened dorso-ventrally and much broader anteriorly than in the middle. Its shape may be seen in fig. 29, which gives an accurate representation of the pair of plates after the first post-embryonic moult (stage 8). The tracheal lumen (*tr. l.*) now extends to near the anterior end, where it is also broader, but since the dorsal and ventral surfaces are practically in contact (fig. 29A) this portion can scarcely function as a respiratory organ at this stage.

In the second place, the lateral tracheal proliferations (*tr. prol.*) have also considerably elongated, but in a lateral direction to form a broad flattened lobe on each side. A portion of the lumen of the trachea is continued into the basal part of this lobe, and I have indicated the lumen by the dotted lines (*tr. l.*), as far as I could trace it with certainty, but there are indications in the sections that the lumen penetrates even further. It is extremely difficult to ascertain the exact shape of the lateral ends of the lobes, as they are wedged in between several other tissues, and it is just possible that they are bilobed and not rounded as I have drawn them. That portion which could be followed with certainty is drawn with plain lines, and the uncertain parts are indicated by the dotted outline in fig. 29.

In the third place, a short basal portion has been added, forming a hollow stalk or pedicel (*ped.*) connecting the whole apparatus with the outer epithelium. This pedicel is supported on each side by a chitinous rod-like thickening (*rd.*) in the form of a fold springing into the lumen from the lateral edges of the chitinous lining and corresponding to the "prolongement chitineux" described by Schimkewitsch ('84, p. 66, Pl. ii, fig. 6) in the adult of *Epeira*.

The small entochondrite (*t.*), which in fig. 28 is attached to the hypodermis near the extreme lateral ends of the spiracle, is now found a long way off from the spiracle. By comparing the two figures it will be seen that the entochondrite has not actually changed its position but that the spiracle (*sp.*) itself has greatly contracted, being now, in fact, less than half its former width, and thus the tissue bounding its lateral ends now comes to lie some distance away from the entochondrite. In shape this entochondrite (*t.*, fig. 29) has greatly elongated. It is broader towards the ends and slenderer just behind the middle and is attached at its posterior end (at *x*) directly to the hypodermis. To the larger anterior portion three muscles are attached, viz. the anterior oblique muscle (*a. ob. m.* 10) and two other muscles, the medial and lateral spinner muscles (*m. sp. m.* 10 and

l. sp. m. 10), which pass posteriorly and attach themselves to the medial and lateral parts respectively of the base of the left anterior spinner. The same muscles are seen in fig. 28. The smaller posterior portion of the tendon is further connected with the lateral edge of the tracheal pedicel by a small transverse column of cells (*tr. m.*), apparently of a muscular nature and plainly corresponding to the little tracheal muscle found by Schimkewitsch ('84, p. 66, Pl. ii, fig. 6)¹ and subsequently also by Lamy (:02, p. 160, Pl. viii, figs. 4, 5) in the adult of *Epeira*. Schimkewitsch considers these muscles to serve the purpose of closing the lumen of the tracheal pedicel, which in the adult, as well as in the young, is strongly compressed dorso-ventrally. The lateral part of the tracheal proliferation lies under the two spinner muscles and the entochondrite, *t.*, and the posterior edge of the proliferation is apparently wedged in between the spinner muscles and the transverse tracheal muscle.

The lumen of the whole trachea at this stage is lined with a smooth but strong cuticular membrane (*cu.*, fig. 29A). The great ventral longitudinal muscles (*v. l. m.* 10) of the tenth somite are stretched some distance above the trachea between the entochondrites *t.* 9 and *t.* 10. The former of these entochondrites is attached as before to the anterior end of the tendinal portion of the trachea (*ec. t.* 9), while the latter lies above the spiracle and is attached to a long hollow entapophysis from the posterior side of the anterior pair of spinners.

After the second post-embryonic moult (stage 9) the tracheæ appear for the first time as a fully functional respiratory organ. In shape they are not much changed, except that the lateral proliferations now branch at the ends into two smaller tracheæ, but beyond these I could not find any other branchlets at this stage. The chitinous lining is now covered (except in the pedicel) with the palisades of hooped (anastomosing) spines, also found in the adult spider, which keep

¹ In the figure the muscle is marked *ep.*, but in the text (p. 88) these letters stand for the chitinous thickening.

the lumen permanently open and allow the air to circulate freely through it.

The anterior (ventral) and posterior (dorsal) walls of the pedicel are close together and lined with a smooth, stout, chitinous membrane, but the two main tracheal trunks are now connected by a spined intertracheal canal of communication, exactly resembling the similar canal already described for the lungs.

I have no other stages between this and the adult form, the chitinous skeleton of which is drawn in fig. 31, and may be readily derived from the post-embryonic stages just described. In fact the only essential difference between the adult form and that after the second moult consists in the presence in the former of a large number of fine tracheal tubules or secondary branchlets, which spring from the main trunks either singly or in clusters, particularly from the ends of the tendinal trunks and of the two branches of the lateral trunks.

The entochondrite (*t. 9*) of the earlier stages is now found attached to the apex of a main tendinal trunk (*m. tr.*), which is not continued beyond the entochondrite in this species except in the form of a bunch of fine tubules.

In *Attus*, therefore, the two main tracheal trunks (*m. tr.*, fig. 31) are serially homologous with the pair of entapophyses or ectodermal tendons (*ec. t.* 8, fig. 20), to which the entochondrites of the ventral longitudinal muscles of the pulmonary and tracheal somites are attached, and are actually homologous with the corresponding entapophyses of the second pulmonary segment of *Tetrapneumonous* spiders.

The lateral basal lobes (*l. tr.*, fig. 31) of the tracheæ are directly derived from the lateral proliferation of the earlier stages, and are serially homologous with the pulmonary sacs of the previous segment, and are to be considered as actually homologous with the pulmonary sacs of the second pair of lung-books of *Tetrapneumonous* spiders.

Critical remarks on the literature.—Schimkewitsch was the first to figure a stage in the development of the trachea of a spider, for in his Russian paper ('86a) he gives a sketch (fig. 29A) of what is evidently the tendinal portion of the trachea (*ect.*) and the entochondrite (*L.* 2) attached to it. I am unable at present to consult his principal paper on the development of Spiders ('87), but apparently Schimkewitsch failed to recognise the tracheal nature of the ectodermal tendon, *ect.*, which he considered to be a provisional structure, as is evident from the following remark in a later paper ('94, p. 210): "Bei den Araneinen, wo das Endoskelet im Abdomen fehlt, entstehen beim Embryo unter den hintern Sehnen provisorische Ectodermfalten, die von mir auf fig. 11, tab. 22 ['87] abgebildet sind." By "Sehnen" the author refers to the entochondrites of the ventral longitudinal muscles.

Simmons ('94) gives two figures of the developing trachea. His earliest stage (fig. 8) is a sagittal section cut at a period when the tracheal appendages are on opposite sides of the embryo (my stage 5). It, therefore, represents a section through the dorsal proliferation of the tracheal sac, and is, as Simmons correctly claims, homologous with the pulmonary sac. On the other hand, his second figure (fig. 9), cut after the reversion, evidently represents the tendinal portion of the trachea, and cannot be the same structure as that represented in fig. 8, as Simmons claims it to be.

Simmons also claims to have found rudiments of the pulmonary folds, and interprets certain undulations on the surface of the embryonic trachea and two in-pushings at its ends (fig. 8) as such, but without, I think, sufficient justification for doing so. Similar undulations may be found in *Attus floricola* (e. g. on the posterior surface of appendage 2 in fig. 5), which certainly bear a superficial resemblance to the pulmonary folds in appendage 1, but these undulations are produced by the mitoses of nuclei lying quite near the surface, and may occur on any part of the body. They have certainly nothing to do with pulmonary folds. Also, the two in-pushings

figured by Simmons do not resemble pulmonary folds, being on the opposite sides of the tracheal tube, and they can hardly be "tracheal twigs" as Simmons suggests, since the lateral tracheæ are, I believe, unbranched in the two forms examined.

The Attus-type and similar types of tracheæ in other spiders.—

The Attus-type of tracheæ has been found in various other genera of Attidæ (Bertkau, Lamy), and is possibly the prevailing type in this family.

A very similar type, not sharply separable from the Attus-type, has been described by Bertkau and Lamy under the name arborescent type of tracheæ, on account of the more frequent branching of the twigs given off by the main trunks. Other differences, according to Lamy, are the presence of a spiral thread in the main trunks, and the prolongation of these trunks into the cephalothorax. Such tracheæ have been found in the Uloboridae (Uloborus, Lamy, :02, fig. 3, Miagrammopes, fig. 5), Prodidomidæ (fig. 26), Zodariidæ (Zodarion, fig. 31), Clubionidæ (Anyphæna, fig. 51) and Attidæ (Ballus, fig. 67).¹

In all these forms the main trunks probably represent the entapophyses, while the small, branched, lateral lobe at the base of each trunk is, no doubt, the rudimentary homologon of a pulmonary sac, exactly as in Attus floricola.

Lamy failed to recognise the homologon of the pulmonary sacs in these lateral lobes in the arborescent and Attus-types of tracheæ, and supposed that here the ectodermal tendons and the lateral trunks (representing the pulmonary sacs) were completely fused together and no longer distinguishable. He also strangely misunderstood my statement on the subject, for he quotes (:02, pp. 257 and 260) me as having said that in the Attidæ the homologon of the pulmonary sac takes no part in the formation of the tracheæ, which are entirely formed of the entapophyses, and he then proceeds to dissent from this view.² My actual statement

¹ The figures referred to are all in Lamy (:02).

² Thus on p. 260 he says: "En tout cas, l'opinion de Purcell sur les

was ('95, pp. 398, 399): "The homologon of the lung is represented in the latter groups [Agelenidæ, etc.] by the lateral pair of tracheal trunks, but in the Attidæ by a mere rudiment in the form of a short lateral process on each side at the base of the two large trunks."¹ There was, therefore, no need to have differed from me as to the presence of the homologa of the lungs.

The Agelena-type of tracheæ and its development.—It was shown long ago, first by v. Siebold ('48) and later by Bertkau ('72, '78) and Lamy (:02), that many families of Dipneumonous spiders (about half of the genera examined, according to Lamy, p. 227) possess a much simpler tracheal system than that which occurs in the Attidæ. This simplified system² consists of four long trunks united behind at the base, as in fig. 21, but without any of the fine secondary tubules found in the Attidæ.

Such tracheæ occur in the Agelenidæ, Clubionidæ, Drassidæ, Argiopidæ, Lycosidæ, Theridiidæ, etc., and their relation to the Attus-type of trachea may be at once seen by comparing fig. 21 (Tegenaria) with fig. 29 (young Attus). Here the tendinal trunks (*m. tr.*) in the latter are obviously equivalent to the medial pair of trunks in Tegenaria, while the lateral branch (*l. tr.*) on each side in Attus is represented by the pair of lateral trunks, which, therefore, are serially homologous with the dorsal proliferation of the embryonal pulmonary sac.

That this is really the case may also be easily shown from the embryological material of *Agelena labyrinthica* in my possession. Shortly before the hatching of the embryo and after the completion of the reversion in this species, the pair of tracheal plates have very much the same form as in

trachées des Attidæ, auxquelles il donne une origine entièrement entapophysaire, ne me semble pas acceptable."

¹ On p. 248 Lamy curiously enough correctly quotes this statement.

² Literature: v. Siebold ('48, p. 535), Leydig ('55, p. 460), Bertkau ('72, '78), Schimkewitsch ('84), W. Wagner ('88, figs. 26, 67, and 68), Vogt ('89, p. 226), Lamy (:00, :01, :02).

Attus floricola (fig. 28) at the same stage (stage 6). They are, however, much further apart, and, therefore, with a wider intertracheal infolding connecting them, the lateral proliferations being also more pronounced. Further, each plate is much thinner in the middle and lateral region than at the base and along the anterior and medial margins.

In embryos one to two days after hatching (stage 7) the tendinal portion of each plate has considerably increased in length and is, like the rest of the plate, very thin, except at the apex, where it rather suddenly swells out and ends in a thick knob to which the entochondrite is attached.

After the first post-embryonic moult (stage 8) the tendinal portion of the trachea has much the same appearance as in the previous stage, except that it has increased in length, but the lateral proliferations have grown for some distance in a lateral direction close to the hypodermal covering of the body and are now provided with a distinct lumen in the form of a very fine canal lined with chitin and communicating with the spiracle. The chitinous lining both of this and of the tendinal portion is smooth at this stage.

At the second moult (stage 9) the trachea assumes its permanent shape. The chitinous lining, except in the pedicel, becomes provided with the usual anastomosing spines and the lateral proliferations increase considerably in length, still growing in a lateral direction. The pedicel and the canal of communication also appear. In fig. 30 (just before the second moult) the hooped spines (*spi.*) of stage 9 have already appeared in readiness for the moult.

Both in this stage and in the previous one the lumen of the lateral proliferation (*tr. prol.*, fig. 30), in its basal region at least (i. e. near the pedicel), is eccentric, lying posteriorly to the axis, the posterior wall of the trachea being much thinner than the anterior wall, which contains nearly all the nuclei. Towards the apex this wall becomes much thinner and the lumen lies practically in the middle. This eccentricity of the lumen is significant of the origin of the lateral trachea, and may be at once understood if we remember that

the thicker anterior wall of the trachea is equivalent to the anterior wall of the pulmonary sac together with the lung-saccules produced by the latter.

Three forms of this type of trachea are mentioned by Bertkau ('72), namely, those having: (1) the two median trunks united at base to a short common tube, as in the Theridiidæ and some Argiopidæ (Bertkau and Lamy); (2) a medial and lateral trunk united at base in pairs on each side to form two short common trunks, as in Tegenaria (fig. 21), this being the usual form, according to Bertkau and Lamy; and (3) the four trunks springing separately from the pedicel, as in some Argiopidæ, e. g. Linyphia (fig. 25).

The resemblance between this third form of tracheal system (fig. 25), in which the lateral trunks at first take a lateral course before running forwards, and the pulmonary system of an Attus (fig. 20) is very striking, and clearly shows the homology of the medial trunks (*m. tr.*) with the entapophyses (*ec. t.* 8), and of the lateral trunks (*l. tr.*) with the pulmonary sacs (*pulm. s.*). The parallel between the spinous intertracheal canal of communication (connecting the median trunks with one another and with the lateral trunks at their base) and the interpulmonary canal of communication (connecting the entapophyses with one another and with the pulmonary sacs) is complete, as may be seen by comparing fig. 24 with fig. 23 (sagittal sections through the lateral region of the canal [*can.*] along the lines indicated in figs. 25 and 20), and fig. 26 with fig. 23B (median sections along the lines indicated in figs. 25 and 20). From these figures it will also appear perfectly clear that the medial trunks of the tracheal system cannot be considered as branches of the lateral trunks any more than the entapophyses of the pulmonary segment are branches of the pulmonary sac.

In the Agelena-type of tracheæ the medial trunks generally take a fairly straight course as far as the ento-

chondrite, where they may either terminate and thus remain comparatively short, as in *Araneus* (*Epeira*), according to Lamy, or they may become longer and be continued beyond the point of attachment on the lateral side of the entochondrite, often winding about for a short or even a considerable distance further before coming to an end (*Agelena*, *Tegenaria*, *Melanophora*, *Pachygnatha*, *Clubiona*, etc.). These long trunks are frequently bifid for some distance from the apex, a character first observed by W. Wagner ('88) in *Lycosa*, and subsequently by Lamy (:02) in several other forms (*Agelena*, *Zora*, *Tibellus*, etc.).

The form with short medial trunks has been carefully studied by Lamy (:02), who was the first to describe the method by which these trunks are attached to the entochondrite. The tendinal trunks, according to Lamy, are produced at their ends into a chitinous fibrous piece which adheres to the entochondrite and is not furnished with a spinous cavity and therefore presents "absolument un aspect entapophysaire ou tendineux" (:01b, p. 178). This fibrous termination was observed by Lamy in most *Theridiidæ* and various *Argiopidæ* (*Linyphia*, *Araneus*, etc.).

The form of trachea with long medial trunks is very widely distributed, but its mode of attachment to the entochondrite has evidently eluded the observation of Lamy, for he nowhere makes any definite statement nor gives any figure regarding this point, except in the case of *Tegenaria*. In this genus the medial trunks are said to terminate at the entochondrite in the same fibrous process which was observed in the *Theridiidæ*, etc. (Lamy, :01b, p. 178), and one of the trunks is figured as ending in such a process (:02, p. 213, fig. 58).

I have examined five adult specimens of *Tegenaria domestica* after treatment with caustic potash, and always found the medial trunks evenly rounded off at the apex and spined internally to the very tip, but without any trace of terminal fibres. At a distance from the apex equal to about two fifths of the whole length I found one or more short,

fibrous, chitinous processes (*hy'*, fig. 42) attached to the medial side of the trunk, which undoubtedly represent the terminal fibres found by Lamy in *Araneus*, etc., and which connect the trachea with the entochondrite. The part of the trachea which is produced beyond the point of attachment is thinner than the part posterior to the entochondrite, and may be either a single tube as in *B*, fig. 42, or it may consist of two equal (left side, fig. 21) or unequal tubes (*br.*, fig. 42) produced by the branching of the main trunk at the insertion of the fibres. In one case each of the branches was again divided so that the tracheal trunk then ended in four separate points. There is no symmetry about this branching, for one side may be branched and the other not, but in all cases the branches are lined internally with hooped spines right up to their tips, differing in this respect from the ordinary secondary tubules of the *Attidæ*, etc., from which such spines are absent.

The only instance which Lamy mentions of a similar tendinous fibre being attached to one side of a medial trunk is the genus *Chorizomma* (:02, p. 219), in which, however, the tracheæ belong to a different type from the one we are now discussing.

It is probable that the various forms of tracheæ with long medial trunks, whether branched or not, described by Lamy in a number of families (*Drassidæ*, *Argiopidæ*, *Thomisidæ*, *Clubionidæ*, *Agelenidæ*, *Lycosidæ*, etc.), all resemble one or other of the variations of *Tegenaria* in their mode of attachment to the entochondrite.

In *Nephila*, which Lamy reckons with the forms with short medial trunks, I observed the tendinous fibres both at the apex and also on the medial side at some distance from the apex. This form may have, therefore, two places of attachment.

In all cases these medial fibres, and a good part of the terminal ones, are certainly nothing else but the intercellular fibres usually produced by the hypodermal cells of an entapophysis to connect the cuticula with the attached entochondrite or muscle (e.g. *hy'*, figs. 32, 36, etc.). They do not

themselves constitute the entapophysis, which is, of course, formed by the entire ectodermal invagination—that is to say, in this case the medial tracheal trunks.

The tracheæ in the Dysderidæ.—These tracheæ, which have been frequently described,¹ were the first found in spiders (by Leon Dufour² in 1834, teste Bertkau, '72, and Lamy, :02), and are of considerable interest from a comparative anatomical point of view. I have myself examined sections of *Segestria*, *Harpactes*, and *Dysdera*.

The tracheal spiracles of the *Dysderidæ* are widely separated, lying in the anterior region of the body a little behind the pair of pulmonary spiracles (text-fig. 3, p. 69), and entirely unconnected with one another. Each leads into a large tracheal trunk, which rises upwards from the spiracle and then runs forwards and breaks up at its anterior end, either in the pedicel of the abdomen or a little behind it, into a large bunch of fine secondary tubules. At the base a shorter posterior trunk projects backwards, and also gives off a number of fine tubules. The chitinous lining of the trunks is provided with spines, which support a spiral thread (*Dysdera*) or an inner perforated tube (*Segestria*).³ To these well-known facts I have to add the following observations :

The segments of the ventral longitudinal muscles belonging to the tracheal somite are very short in this family, like the somite itself, and the entosternite is attached on the medial

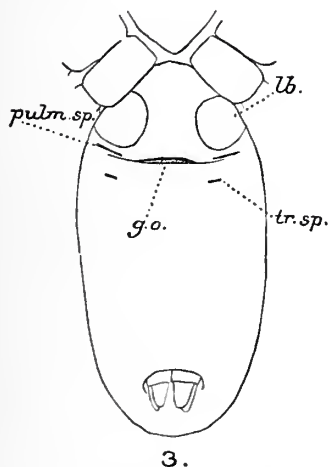
¹ Literature: Dugès ['36, '49; v. Siebold ('48, p. 535) also cites the following of the year 1835: 'Feuill. Acad. des Sci. Séance du 9. Févr.,' also Froriep's 'Notizen,' xliii, p. 231, also 'Ann. Sc. Nat.,' vi, p. 183], Bertkau ('72), MacLeod ('80), Lamy (:02).

² I am unable to find the reference to this paper, unless it be 'le Temps,' No. 1942, cited by Menge ('51, p. 22), which, however, v. Siebold ('48, p. 535) accredits to A. Dugès, both authors giving the year 1835, and not 1834.

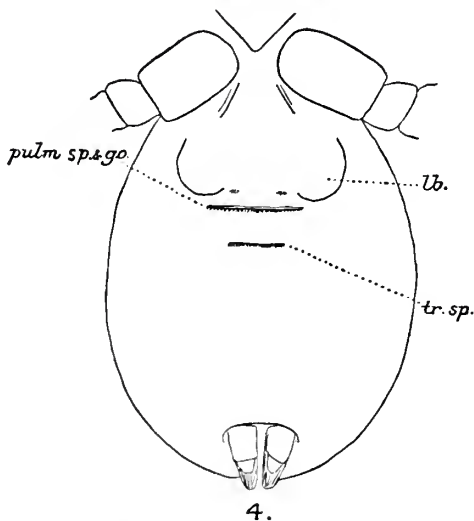
³ Bertkau ('72) states that in *Segestria* these spines do not anastomose, but Lamy (:02, p. 183, fig. 23) has since shown (and I can corroborate his statements) that they certainly anastomose at their ends, forming an inner, fenestrated, chitinous tube. In *Harpactes* the anastomosing branches of the spines form a simple network only.

side of the base of each tracheal pedicel. In *Segestria* the ectodermal area of attachment is drawn out in a mediad direction in the form of a short, flat, unspined pouch (*ec. t.* 9, figs. 32 and 33), which opens into the short, smooth, flexible pedicel (*ped.*) connecting the rigid outer cuticula (*cu.*) with the spinous main trunk (*tr.*) of the trachea. This entapophysis is not respiratory, and the entire trachea is to be regarded as homologous only with the embryonic pulmonary

TEXT-FIG. 3.



TEXT-FIG. 4.



Segestria senoculata, ♀. *Argyroneta aquatica*, ♀.

Ventral surface of abdomen.—*pulm. sp.*, pulmonary spiracle; *tr. sp.*, tracheal spiracle; *lb.*, operculum of lung-books; *g. o.*, genital opening; Magn. 12.

sac and its proliferation, as I have already stated in a previous communication ('95). That this must be the case may fairly be concluded from the position of the pair of spiracles (text-fig. 3) corresponding to the second pair in Tetrapneumonous spiders (text-fig. 2, p. 52) and from the position of the entapophysis on its medial side. Lamy (:02) has also expressed himself in agreement with this view, which differs entirely from that of Bertkau ('72), who considered the short posterior

trunks only to be equivalent to the lateral trunks in other spiders.

The tracheæ in *Argyroneta aquatica*.—The highly-developed tracheæ¹ of this water spider are very peculiar. The two main branches, as Bertkau ('78) showed, have a common spiracle, which opens far forwards (text-fig. 4), close to the pulmonary spiracles. The remarkable point about these tracheæ is the circumstance that they lie entirely on the medial side of the longitudinal ventral muscles. The segment of this muscle belonging to the tracheal somite is very short, corresponding to the anterior position of the spiracle, and it is stretched between two large entochondrites, the posterior of which is attached to the upper surface of a short basal process on the lateral side of the tracheal trunks. This process, which is figured by Lamy (:02, p. 212, fig. 56), is provided in its cavity with spines, like the main trunks, and gives off at its apex a number of fine tracheal tubules (the lateral bunch of tracheæ described and figured by Menge ['51, *dd*, Pl. i, fig. 7] and Lamy).

The process is flattened dorso-ventrally and corresponds to the tendinal trunk of other Dipneumonous spiders. The two large main trunks appear to be outgrowths from the medial side of each very short tendinal trunk. They are joined at their bases by an intertracheal fold provided with the usual spinous canal of communication. On the posterior side of each main trunk, near its base, is a transverse out-folding of the tracheal wall forming a deep spinous groove on the inside of the trachea, connecting the canal of communication with the lateral or tendinal branch. This transverse folding, which was described by Lamy (:02) as an abdominal trunk resembling that in *Segestria*, also gives off numerous tubules, which, together with another group just below, springing directly from the main trunk, form the posterior bunch of tracheæ figured by Menge ('51, *ee*, Pl. i, fig. 7).

In *Argyroneta*, therefore, the entire tracheal

¹ Literature: Grube ('42), Menge ('51), Bertkau ('78, p. 384), MacLeod ('80, '84), Lamy (:02).

system appears to be derived from the tendinal portion of the trachea, and there is no distinguishable trace left of the lateral trunks, which may be homologised with the pulmonary sac.¹ This leads us to the conclusion that the tracheal systems of *Argyroneta* and the *Dysderidæ*, although superficially closely resembling one another, are yet apparently not homologous structures.

The tracheæ in the Scytodidæ, Palpimanidæ and Filistatidæ.—The tracheæ of these three small families possess a peculiar interest, inasmuch as Lamy has shown that their medial trunks are non-respiratory and serve solely as entapophyses for the attachment of the entochondrites. Bertkau ('78) observed that the medial trunks were reduced to an unpaired median rudiment in *Scytodes*, only the lateral ones being developed, but our knowledge of the tracheæ in the other forms is due to Lamy (:00, :01b, :02).

The most interesting is the tracheal system of *Filistata*, of which I reproduce Lamy's figure (:02, p. 173, fig. 12), as I have no material of this family at my disposal. Here, according to Lamy's description, the spiracle is very broad and placed about midway between the interpulmonary fold and the spinners. The two short lateral trunks (*l. tr.*) are pointed sac-like and of the simplest form, exactly as a pulmonary sac would appear if it lost its saccules. The four trunks are connected at base by an intertracheal fold with spines in its deepest part (which no doubt forms a canal of communication). The two entapophyses (*ec. t. 9*), too, have some internal spines in their basal part, but are otherwise unspined, while their free ends are jagged and tendon-like. If we compare this text-figure with the figure of the pulmonary system of *Attus* (fig. 20) and leave the saccules out of account, the parallel between the two

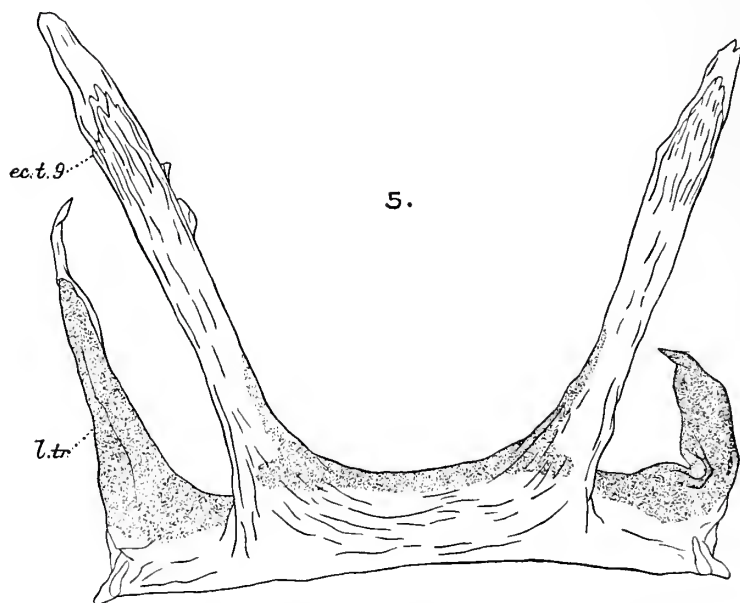
¹ MacLeod's ('82, p. 785, and '84, p. 29) view that the trachea of *Argyroneta* is nothing else than the dorsal chamber of the second pair of lung-books of a *Mygale*, enormously developed, is certainly incorrect.

respiratory systems appears complete and their homologies almost self-evident.

In the *Palpimanidæ*, according to Lamy, the medial trunks are separate at least at their apex, while in the *Scytodidæ* they are confluent to the apex and form a single median trunk.

I have examined preparations in caustic potash and sections

TEXT-FIG. 5.



Filistata capitata Hentz. Tracheal apparatus (after Lamy). *ec. t. 9.* entapophysis; *l. tr.*, lateral tracheal trunk. Magnified 100.

from both families. The long, lateral trunks are connected by a canal of communication (*can.*, fig. 38), lined internally with hooped spines (*spi.*), which also spread into the basal part of the median trunk. This latter is unpaired, and although in my examples of *Palpimanus* there are indications of a bifurcation at the apex, it is not nearly so prominent as in the species figured by Lamy (:02, p. 187, fig. 29).

The median trunk is flattened dorso-ventrally and hollow

internally nearly to the apex, but its chitinous lining is plainly much too thick to allow it to be used for respiratory purposes. In *Scytodes* by far the greater portion of this chitinous lining is smooth internally (figs. 38 and 39), only a small part quite at the base being spined (*spi.*, fig. 38), but in *Palpimanus* nearly one half is lined with hooped spines (fig. 37A). In both genera the greater part of the unspined portion of the entapophysis is in contact with the entochondrites (*t.* 9, fig. 37).

The histological structure of these tracheal entapophyses and of those of the pulmonary segment of *Attus* is quite similar. The section through the basal half of the tracheal entapophysis of *Palpimanus* (fig. 37A) should be compared with the spinous part (*spi.*) of the pulmonary entapophysis given in fig. 23A (in the latter the matrix is not drawn in), while fig. 37 of *Palpimanus* is comparable with fig. 23 of *Attus*, both passing through the places of attachment to the entochondrites, *t.* 9 and *t.* 8. The same fibrous hypodermis (*hy.*) and flattened smooth cuticula (*cu.*) is observable in both figures.

In his description of *Palpimanus gibbulus* Lamy says there are two short medial apophyses without a spinous lining (:02, p. 188), but his figure clearly shows that the two trunks are confluent for the greater part of their length and separate only towards the apex. Lamy evidently considers the confluent portion to be part of the vestibule. In other places, too (:01b, p. 178; :02, p. 174), he states that in all these forms the medial trunks are reduced to the unspined, terminal, tendinous (i. e. fibrous) part found at the end of the medial trunks in *Epeira*, etc., by means of which the attachment to the entochondrite is effected, while the whole portion of the trachea in *Epeira* between the entochondrite and the vestibule are said to be absent in *Palpimanus*. I cannot consider this view to be quite correct, for the entire median process in *Palpimanus* and *Scytodes*, including the unpaired part in the former and the spinous portion in both, constitutes the entapophyses, and the spinous portion lying

between the entochondrite and the vestibule is homologous with the much longer but corresponding portion of the medial tracheal trunks in *Araneus*, *Tegenaria*, etc.

Thus, the medial trunks in *Filistata*, *Palpimanus* and *Scytodes* are homologous with the entire medial trunks in *Araneus*, etc., and not merely with their unspined, fibrous, apical portion, as Lamy suggests.

The unspined portion of the medial trunks in *Filistata*, *Palpimanus* and *Scytodes* may well be compared to the tracheae of a young spider previous to the second moult (stage 8), while this organ is still in its primitive spineless condition. (Compare the transverse section, fig. 29A, of the medial tracheal trunk of a young *Attus* with that of the cuticular lining of the entapophysis of *Scytodes* given in fig. 39.)

In the pulmonary segment the unpaired median entapophysis of a *Scytodes* has its exact parallel in the unpaired median entapophysis of such forms as *Lycosa Darlingi*, described on p. 51. We thus see all the variations of the pulmonary entapophyses repeated in the tracheal segment.

IX. THE ENTAPOPHYSES OF THE THIRD AND FOURTH ABDOMINAL APPENDAGES (THE SPINNERS).

These tendons are unconnected with the respiratory organs and need only be briefly described. They arise at a very early stage, being formed out of the post-appendicular grooves (*gr.*, fig. 4), which appear behind the third and fourth pair of abdominal appendages shortly before the beginning of the reversion (stage 1). At the time of the appearance of the first pulmonary furrows (stage 2) these grooves have deepened and become more pronounced (figs. 5 and 5A), and they may be easily followed through all the later stages (fig. 6).¹ At the end of the reversion they form invaginations (*ec.*

¹ I may point out that no trace of a lateral proliferation corresponding to that of the pulmonary and tracheal sacs is ever found in connection with these grooves.

t. 10 and 11, fig. 41), which may exceed that of the proctodæum (*proc.*) in size. After moulting they form internal cones or processes with a chitinous axis situated at the posterior inner angle of the anterior and posterior spinners respectively. At the stage of fig. 29 (stage 8, after the first moult) the entochondrite (*t.* 10), to which the anterior of these entapophyses is attached, is placed just over the tracheal spiracle, but is, of course, not attached to it.

The chitinous skeletons of the entapophyses of an adult *Tegenaria* and their relation to the anterior and posterior spinners are shown in fig. 21. These spinners are, of course, the third and fourth abdominal appendages, but the middle pair of spinners (*m. spin.*) do not, according to Jaworowski ('95), correspond to a pair of appendages and have consequently no entapophyses. An entochondrite of the longitudinal muscles is attached to the anterior part of each of these entapophyses, the posterior of the three well-known pairs of large abdominal entochondrites¹ described by Schimkewitsch ('84, p. 38) and others being that (*t.* 10) which is attached to the entapophyses of the anterior pair of spinners.

The four pairs of serially homologous entapophyses (*ec. t.* 8-11) may all be seen in fig. 21. They are, of course, connected on each side by a longitudinal muscle, and the positions of the four intermuscular tendons (*t.* 8-11) are indicated in brackets. This figure may, therefore, serve to give a general idea of the inter-relationship of all these tendons of ectodermal (*ec. t.* 8-11) and mesodermal (*t.* 8-11) origin.

X. GENERAL CONCLUSIONS.

The embryological data furnished in the preceding pages will, I believe, enable us to arrive at definite conclusions with regard to certain questions concerning the phylogenetic

¹ These three entochondrites, marked *l2*, *l1*, and *l3* in Schimkewitsch's Pl. vii, fig. 1, correspond to *t.* 8, *t.* 9, and *t.* 10 respectively in my fig. 41.

origin of the tracheæ, as well as of the lung-books in *Araneæ*.

The origin of the tendinal or medial tracheal trunks in *Araneæ*.—As the pair of ventral longitudinal muscles is a very primitive structure, and must originally have been attached to the outer hypodermis, it follows that the tracheal nature of the tendinal or medial tracheal trunks must be a secondary character, for if this were not the case we should have to assume that all the ectodermal areas of attachment of the ventral longitudinal muscles were originally derived from tracheæ, since they are all serially homologous, but this would be an absurd supposition and quite contrary to the facts of embryology and comparative anatomy.

I have also already pointed out that these medial trunks cannot be considered as branches of the lateral ones, nor does the embryological evidence show that they are otherwise than independent metamorphosed entapophyses united at their base with the lateral trunks by an intertracheal fold and canal of communication, exactly in the same way as the entapophyses of the pulmonary segment are united with the pulmonary sacs by an interpulmonary fold and canal of communication. The independent nature of the tendinal trunks is observed in the adults of such forms as the *Attidæ* (fig. 31), owing to the partial fusion of the rudiments of the lateral trunks with the base of the medial ones, but it is clear enough in most other forms. Even in such forms as *Segestria*, *Scytodes*, and *Palpimanus*, where the entapophyses have not been converted into tracheæ, they remain attached to the smooth pedicel at the base of the lateral tracheæ (*Segestria*, fig. 32) or to the spinous canal of communication uniting the two lateral trunks (*Scytodes* [fig. 38], *Palpimanus*), and do not shift their position on to the spinous part of these trunks. These forms, therefore, do not provide us with any grounds for supposing that the spinous parts of the medial trunks in other spiders have originated as outgrowths from the spinous part of a lateral trunk. In fact, we have no other alternative, in view of

both the embryology and comparative anatomy, but to consider the medial trunks of the tracheæ as equivalent in their entirety to metamorphosed entapophyses.

It is, moreover, a common feature in the Arachnida for the ectodermal areas of attachment of various muscles to be invaginated into the body in the form of pouches or tubes for the purpose of serving as tendons, as, for instance, the entapophyses (*ec. t.* 10 and 11, fig. 21) of the two following abdominal segments already described.

In order that an ectodermal tendon may become converted into a trachea it is only necessary that it should be hollow and sufficiently thin-walled, with free access of air to its interior, and that it should lie in blood or tissues requiring aëration. It is also evident that a tendinal trachea must have existed first as a simple entapophysis, since it could not possibly function as a trachea until after it had attained a tubular form. The entapophyses could not, therefore, have been originally produced for respiratory purposes.

In the case of Araneæ I have already sought to explain the elongated tracheal entapophyses by the great elongation of the ninth somite, and since the tubular entapophyses so produced are hollow and lie in the large ventral blood sinus (*v. sin.*, figs. 41 and 43) we have here all the conditions necessary for their conversion into a trachea. For it is well known that the blood passes from this sinus to the lung-books and thence to the heart, and that the sinus, therefore, contains venous blood requiring aëration (Blanchard '49, '50, Claparède '63, Schneider '92, etc.).

In the Tetrapneumonous spiders and in some Dysderida (Segestria) we find the rudiments of the entapophyses of the ninth segment in the form of shallow depressions (*ec. t.* 9, text-fig. 2, p. 52) or pouch-like invaginations (*ec. t.* 9, fig. 32), already described on previous pages. These rudiments have no respiratory function, and if they were to approach near to the median line and be united at base by an intertracheal infolding we should obtain the conditions found in Filistata,

etc. (text-fig. 5, p. 72), and we have only to further imagine these entapophyses lengthened and to become thin-walled and provided internally with spines throughout in order to convert them into the tendinal tracheæ of other Dipneumonous spiders. It is evident that the condition in the Tetrapneumonous spiders, at any rate, is a primitive one, on account of the other primitive characters of this group, but the possibility of a reversion from an elongated tracheal tendon back to a very short one must be borne in mind, and may, perhaps, occur in some Dipneumonous spiders in which the ninth somite has secondarily become shortened again. I do not think that this has been the case in the Dysderidæ, however, on account of the primitive position of the tracheal spiracles (text-fig. 3, p. 69) and other primitive characters in this family, but in *Argyroneta* (text-fig. 4, p. 69) I believe there is every probability that the spiracle was once more posterior and has subsequently shifted forward again to suit a newly acquired, aquatic habit. This would account for the fact that, although the actual tracheal entapophyses are extremely short, they are lined with the usual anastomosing spines and provided with a large medial outgrowth. This outgrowth may originally have been merely a medial prolongation of the tracheal entapophysis beyond the entochondrite, and when the spiracle moved forwards and the entapophysis shortened, its medial prolongation may have increased in inverse proportion, so as to maintain the effectiveness of the entire trachea as a respiratory organ.

It would certainly appear that the tendinal trunks are more effective breathing organs than the lateral trunks are, probably on account of the position of the former in the great ventral sinus of venous blood. For we frequently find the tendinal trunks very strongly developed, and the lateral ones correspondingly reduced to a mere rudiment (*Attidæ*) and sometimes apparently to vanish altogether (*Argyroneta*).

The origin of the lateral tracheal trunks in *Aranææ*.—The second question to be considered is whether the pair of lateral tracheæ of Dipneumonous spiders was derived from the second

pair of lung-books of Tetrapneumonous forms or whether the reverse was the case.

That the lateral tracheæ are serially homologous with the pulmonary sacs of the preceding somite and, therefore, homologous with the same part of the lung-books of the ninth somite in Tetrapneumonous spiders, cannot, I think, be disputed, although the embryology of the latter group is not yet known.

In deriving the lung-books from tracheæ the simplest theory and the one that has been usually adopted by those who favoured this view, is to consider the pulmonary sac or antechamber to represent the main trunk of a trachea and the saccules merely modified lateral branches arranged in a single row and flattened by mutual pressure.

A very serious objection to this view lies in the appearance of the two oldest pulmonary saccules on the embryonic appendages quite outside of the pulmonary sac. These two saccules cannot be branches of the main trunk, and in order to account for their presence we should have to assume that they themselves at one time each represented a separate tracheal trunk. This, however, could hardly have been the case, since all the saccules are formed in the embryo in exactly the same manner (apart from their position out of or within the sac) and should, therefore, have exactly the same phylogenetic origin.

Another view based by Jaworowski ('94) on embryological grounds and adopted by Bernard ('96, p. 375) on theoretical ones is to the effect that the lung-books arose by horizontal folds in the basal part of a vertical tracheal trunk. Here also the appearance of the two oldest saccules, entirely outside of the pulmonary sac, is too strong an argument against our acceptance of this theory, which, moreover, Jaworowski has failed to prove embryologically, as I have already pointed out on a previous page (p. 33).

In fact the only way we can derive the saccules of lung-books from tracheal tubes which appears to me at all feasible is to assume that an ancestral form of the Araneæ possessed

abdominal appendages, on the posterior side of which were a number of separate tracheæ arranged in a row, and that these appendages were sunk into the body in later forms. The tracheated appendages of such an ancestral form would, in fact, be very similar to one of the transitional stages which Kingsley assumes for his theory of the origin of lung-books from gills (p. 27), but it would be totally different from anything actually found in the tracheal system of existing spiders.

From purely embryological considerations, therefore, and quite apart from the branchial theory of the origin of the lung-books, we have to assume that the pair of lateral branches of the tracheæ of the ninth somite in Dipneumonous spiders must have been derived from the pulmonary sac and not the reverse. This conclusion is, moreover, strongly confirmed by the fact that the Tetrapneumonous spiders, and particularly the remarkable genus *Liphistius*, are more primitive in their other characters than are the Tracheate spiders.

The origin of the secondary tracheal tubules.—The third question is the nature of the tracheal branchlets, those fine tubules (*tr. tub.*, fig. 31) given off by the main trunks in certain forms (*Attidæ*, *Dysderidæ*, *Argyroneta*, etc.). It is usual to consider these as homologous with the saccules of the lung-books, whatever view¹ may be taken of the origin of the latter. I think, however, that this homology is, for the most part, erroneous.

Since the pulmonary saccules occur only on the anterior side of the pulmonary sac, we should expect to find the tracheal tubules on the corresponding surface of the lateral tracheal trunks, but this is by no means the case.² Thus, in

¹ Except, however, Jaworowski and Bernard.

² In the remarkable anterior pair of tracheæ of the Apneumonous Family *Caponiidæ*, described and figured in Simon ('Hist. Nat. Araign.' 2^e éd., i. pt. ii. pp. 326, 327, figs. 294 and 295, 1893) after Bertkau, the tubules are nearly all placed, however, on the anterior side of an oval ante-chamber, and here, no doubt, do correspond to pulmo-

the *Dysderidæ*, in which the entire tracheal system is probably derived from lung-books, we find the tubules arising in a dense cluster from the apex of the elongate trunks and from a small basal branch on the posterior side, but none from the anterior or under surface of the trunks. Further, in all tracheæ of the *Agelena*-type, which is that of the majority of the *Dipneumonous* families, the lateral trunks have no secondary branchlets at all.

On the other hand we find these tubules at various places on the tendinal trunks in the *Attidæ* and other groups (fig. 31), which shows that the tubules may arise anywhere on a tracheal trunk, when required, and quite independently of the pulmonary saccules, since in this case they could not have been derived from the latter. In *Attus floricola* there is no embryological evidence that the tubules of the lateral tracheal branches have anything to do with pulmonary saccules, for whereas these latter commence to form in the pulmonary segment at an early embryonic stage the tubules do not appear until long after the young spider has been hatched. It is, however, conceivable that the earlier lung-saccules may have been entirely suppressed in the tracheal segment, so that only the post-embryonic lung-saccules reappear as secondary tracheal tubules in certain cases, and the possibility of the anterior terminal tubules of the *Dysderidæ* and those of the lateral lobes of the *Attidæ* being of this nature must be borne in mind.

Bertkau ('72, '78) attempted to utilise the presence or absence of secondary tubules as the basis of a system of classification, but Lamy (:02) has shown that this character has little value for this purpose, since within the same family some forms may be provided with tracheal tubules, while closely related forms are entirely without them.

The origin of the lung-books in Arachnids.—A fourth question in connection with this subject is whether the lung-books of nary saccules, since the ante-chamber is doubtless that of the pair of lung-books which the tracheæ have replaced. I examined sections of *C. spiralifera*.

the Arachnids were derived in the first instance from tracheal books or from gill-books.

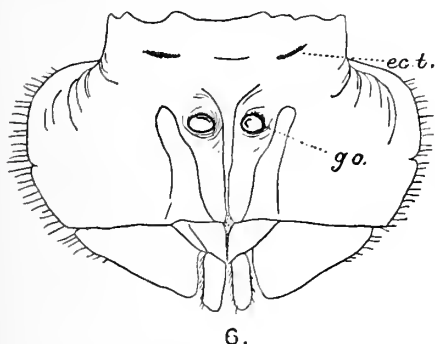
I have endeavoured to demonstrate in a preceding paragraph that, since all the lung-saccules within the pulmonary sac precisely resemble in their formation and structure the two oldest which appear outside of this sac, all the saccules must have had the same phylogenetic origin and must consequently all have originally been upon the posterior surface of the abdominal appendage. The question, therefore, is whether the saccules of this primitive appendage in the ancestral Arachnid were tracheæ or whether they were produced from sunken-in gill-lamellæ. Whereas the appearance of a number of tracheæ in such a position seems most improbable, the arguments in favour of the branchial origin appear overwhelming. Most important amongst these, next to the embryological evidence, is the undoubted general agreement and affinity between *Limulus* and Arachnida, first pointed out by Straus-Durckheim and v. Beneden, and afterwards so ably demonstrated by Ray Lankester. The embryological side of the question and the probable manner in which the transition from gill-books to lung-books may have taken place has already been fully discussed (pp. 17-44) and need not be considered again.

I shall only introduce here two figures of the abdominal appendages of *Limulus* for comparison with the pulmonary segment of a spider drawn in fig. 20. The appendages of the genital segment (text-fig. 6), which are homologous with those of the pulmonary segment, have no gill-books, but possess the pair of genital openings (*g. o.*), which would lie between the gill-books, if the latter were present. Text-fig 7 represents a branchiate appendage, and it will be seen that if the gill-leaves were sunk into the appendage and the latter into the abdomen, we should have exactly the condition found in a spider (fig. 20). The large entapophyses (*ec. t.*) shown in the text-figures are not, however, homologous with those of the pulmonary segment (*ec. t.* 8, fig. 20).

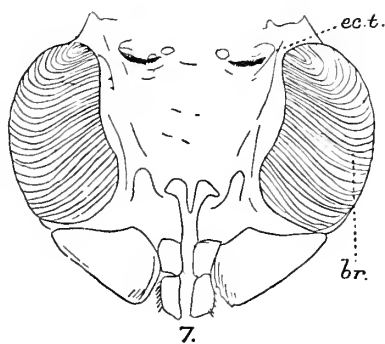
The endeavour to derive all tracheæ in Arthropods from a

common origin has no doubt weighed considerably against the acceptance of the branchial origin of lung-books, but this should not be the case in view of the undoubted diphyletic origin of the tracheæ in Araneæ, which, I think, I have sufficiently demonstrated. Further, in one and the same spider both parts of the tracheæ, although of different origins, have exactly the same histological structure, hence similarity of structure in the fully developed tracheæ does not mean similarity of origin. I mention this here expressly, since this similarity of structure has been used as

TEXT-FIG. 6.



TEXT-FIG. 7.



TEXT-FIGS 6 and 7.—Appendages of the genital segment and a pair of abdominal branchiate appendages of *Limulus*, seen from behind (after Ray Lankester). *g. o.*, genital openings; *br.*, gill-book; *ec. t.*, external opening of an ectodermal tendon.

an argument in favour of the monophyletic origin of all tracheæ.

The appearance in spiders of tracheæ as newly acquired organs derived from two separate and distinct sources simultaneously with the occurrence of other well-developed organs of respiration clearly shows how readily tracheæ may be acquired.¹ Why, then, should they not have originated

¹ Pocock ('93), who was of opinion that tracheal tubes replaced lung-books at least twice in the group Arachnida, viz. in the Dipneumones and in the Pseudoscorpiones, remarks (p. 17): "The fact that these tubes have been developed twice in the same group bears very strong evidence as to their efficacy as breathing organs. They

equally readily over and over again in the Arachnida, and particularly in so large and diversified an assemblage as the Tracheata? Thus in the Solifugæ the thoracic tracheæ, which open at the base of the third pair of legs and have always been an unexplained anomaly in view of the branchial theory,¹ may easily have originated from the entapophyses of some muscle. The same remark applies to the occurrence of the remarkable pair of tracheal spiracles discovered by Hansen ('93, p. 198), and subsequently confirmed by Loman ('96) on the tibiæ of the four pairs of legs in the Phalangiidæ.²

I do not mean to imply that these abnormal tracheæ were must, in fact, be better adapted for their purpose than the lung-book tracheæ." This remark of Pocock's may possibly explain why such highly segmented forms as the Solifugæ have highly developed tracheæ only, since the extraordinary activity of the members of this group would require the presence of the most effective breathing organs. Bernard ('96, p. 374) mentions that these are the only Arachnids in which the primitive tracheal tubes anastomose (as in the Insecta), and to this I may add an observation which I have often made on living Solpugidæ, which is that regular and pronounced respiratory movements are observable in the middle part of the body, especially after the animal has been running. Similar movements have not hitherto, so far as I am aware, been recorded for any air-breathing Arachnids (see Plateau, '86).

¹ Bernard ('92, p. 521), for instance, remarks that the presence of tracheæ on the cephalothorax in Arachnida is "one of the principle difficulties in the way of those who would deduce the Arachnidan abdominal tracheæ from embedded gills. . . . It compels us, for instance, to assume that the cephalothoracic tracheæ have had an entirely different origin, so that . . . it is necessary to assume that the same structures, tubular tracheæ, have had two independent origins in the same animal! . . . there is absolutely no difference between the tracheæ which open through the large stigmata of the thorax and those opening through the more insignificant stigmata in the abdomen [in the Solifugæ]. It is difficult to believe that they had a separate origin. The embedded gill theory must, I think, definitely give way before some simpler theory, such as that here put forward." So also Weissenborn ('87, p. 114).

² It is interesting to note that Loman found these spiracles absent in very young Phalangiidæ.

probably muscular tendons. They may have had any other origin. Thus J. Wagner ('94, p. 126), who has investigated the embryology of Acari and admits the branchial origin of lung-books, explains the cephalothoracic tracheæ of Acari and Solpuga by deriving them from unicellular hypodermal glands, such as are found in water mites; while Börner (:02, pp. 455, 461, and 463) considers it very probable that the spiracle on the prosoma is that of the genital segment displaced forwards. Further, Ray Lankester's suggestion that tracheæ may have arisen by the tubefaction of mesodermal strands may apply.

The primitive nature of the lung-books in comparison with the tracheæ within the class Arachnida is in full agreement with the teachings of the comparative anatomy of other organs. Thus we find only lung-books in the highly segmented orders Scorpiones and Pedipalpi, tracheæ in the orders with more concentrated bodies, Opiliones and Acari, while in the Araneæ the more primitive Tetrapneumones have lung-books only, the more highly specialised Dipneumones tracheæ as well. The Solifugæ, however, which are highly segmented, have tracheæ only (see footnote on p. 84).

Bernard's theory that tracheæ have arisen from bristle-sacs of Chætopod Annelids cannot be maintained for a moment as an explanation of the lung-books or tracheæ of Araneæ in spite of the resemblance which the ectodermal tendons of the tracheal segment in my fig. 41 bears to the bristle-sac with its two oblique muscles figured by Bernard ('92, text-fig. 1, p. 512). Indeed, Bernard does not attempt to derive entapophyses from bristle-sacs, and the rudimentary spiracles (vestigial stigmata) which he claims to have found in Pseudoscorpiones ('93a, p. 422, and '93b, p. 26) and Pedipalpi ('94, p. 151) are always placed by him on the lateral side of the depressions caused by the dorso-ventral muscles of the abdomen.

The homologies of the pulmonary segments in Arachnids.—On the accompanying page I have given a table representing

Table showing Homologies of the Abdominal Appendages and their Derivatives.

Somite.	Scorpiones.	Tetrapneumonous Araneæ.	Dipneumonous Araneæ.	Pedipalpi.	Japanese Limulus.
VIII	Genital operculum	First pair of lung-books	Pair of lung-books ¹	First pair of lung-books	Genital operculum.
IX	Pectines	Second pair of lung-books	Lateral tracheal trunks ²	Second pair of lung-books	First pair of gill-appendages.
X	First pair of lung-books	A pair of spinners	A pair of spinners	—	Second pair of gill-appendages.
XI	Second pair of lung-books	A pair of spinners	A pair of spinners	—	Third pair of gill-appendages.
XII	Third pair of lung-books	—	—	—	Fourth pair of gill-appendages.
XIII	Fourth pair of lung-books	—	—	—	Fifth pair of gill-appendages.

¹ Represented in the Caponiidae by the anterior pair of tracheæ.² Represented in the Dysderidae probably by the entire tracheal system, and in Argyroseta not at all.

the homologies of the abdominal appendages of the eighth to thirteenth somites in *Limulus* and the three pulmonate orders of Arachnida, based upon the most recent embryological researches. The segmental homologies given in this table agree with that of the same six somites given by Börner (:02, pp. 456, 457), and may be taken, so far as the pulmonate Arachnida are concerned, as sufficiently established, whereas the homologies of these segments in most of the Tracheate orders of Arachnida cannot be considered as satisfactorily established, since the necessary embryological evidence is wanting and that afforded by comparative anatomical research insufficient.

The most important point in connection with this question is the position of the genital opening.

(1) *Araneæ*.—I have shown in a previous paper ('95) that the genital ducts in *Attus floricola* are formed out of part of the cœlom of the pulmonary somite and open externally into the interpulmonary (epigastric) in-folding, which lies between the eighth and ninth somites. The genital segment in Dipneumonous spiders is, therefore, identical with the first pulmonary segment, which has been shown to be the eighth post-oral by all the most recent investigators (Kishinouye, '90; Simmons, '94; Jaworowski, '94; and myself, '95).

(2) *Scorpiones*.—Brauer ('95) has clearly shown that the seventh somite in the scorpions had been overlooked by previous authors, and that the genital operculum belongs to the eighth somite, the pectines to the ninth, and the four pairs of lungs to the tenth to thirteenth. Pereyaslawzewa (:07) also places the four pairs of lung-books in the tenth to thirteenth somites (pp. 174–176).

The homologies of the abdominal appendages in scorpions and spiders given in the table on p. 86 may, therefore, be considered as fully established by embryological evidence.

We have thus the remarkable fact, which I pointed out before ('95), that none of the lung-books in scorpions are actually homologous with the two pairs in spiders, and further, the two pairs of lung-books in spiders

are represented by external appendages in the adult scorpion, and the two anterior pairs of lung-books in the latter by external appendages in the adult spider. Now I cannot imagine that the pectines of scorpions could have been derived from appendages which had already sunk into the abdomen and been converted into lung-books, and the converse, that these external organs, after having lost their branchial nature and acquired new functions could ever have been converted in lung-books, is equally improbable. I consider, therefore, that the lung-books of the scorpions and those of the spiders must have been derived from branchiate appendages quite independently of each other, and that the terrestrial Arachnids are not monophyletic but must have had at least a diphyletic origin from primitive aquatic Arachnids with six pairs of abdominal branchiate appendages on the eighth to thirteenth somites.¹

Laurie ('93) has expressed a similar opinion but based on palæontological grounds, that the lung-books in scorpions arose independently of those in other Arachnids.

(3) Pedipalpi.—It has been recently shown by Schimkewitsch (:06) that in the embryo of *Thelyphonus* the lung-books belong to the second and third abdominal somites (p. 43), while the genital opening is found between these two segments (pp. 63, 64), that is to say, exactly as in the *Araneæ*.²

¹ It is interesting to note in this connection that Schimkewitsch ('94, p. 207) discovered in the embryos of a scorpion on each side on the genital operculum three to four teeth (Warzen, *Km.*, fig. 12) which were formed on the same plan as those of the pectines but vanished again before birth. Schimkewitsch thinks it very probable that the genital operculum was once a sense organ like the pectines, and asks whether both were not once gills?

² Hansen ('93, p. 165) had previously pointed out that in *Thelyphonus* the first abdominal sternite should be sought for in the small sclerite at the anterior end of the abdomen, so that the large anterior sternite, which covers the genital opening and the first pair of lung-books, would, according to Hansen, belong to the second abdominal

Laurie and Gough, who examined embryos of Phrynids, are not quite clear as to the segmental position of the lung-books. Laurie ('94, p. 34) states that the first pair belongs either to the first or second abdominal somite, while the second pair belongs to the third somite. According to Gough (:02, p. 616) the lung-books belong to the first and second abdominal appendages, but the author does not say to which somites they belong. Pereyaslawzewa (:01), on the other hand, describes distinct paired appendages on each of the first five abdominal somites, the lung-books being formed from the third and fourth pairs (p. 194).

In view of the definite statements made by Schimkewitsch, as well as of the anatomical evidence afforded by the adult (see footnote on preceding page), and of the close relationship which the Pedipalpi bear to the Araneæ, we may accept as certain that the lung-books in the former group belong to the second and third abdominal segments, i. e. the eighth and ninth post-oral somites, and that the genital segment is the second and not the first of the abdomen, as stated by Laurie ('94). This would make the lung-books in the Pedipalpi directly homologous with the corresponding ones of the Araneæ, as represented in the table on p. 86.

I consider that the pulmonate Arachnids comprise two distinct groups, which have separately originated from branchiate ancestors, namely, (1) the Scorpiones, and (2) the Araneæ and the Pedipalpi. To the latter phylum some, if not all, of the remaining orders of tracheate Arachnida may perhaps be added, but I shall not at present enter further into the relationships of these other orders.

Pocock ('93) has already expressed the opinion that the Scorpiones, although the most primitive of all terrestrial Arachnida, could not have been the ancestors of any other orders of Arachnida, because the useful tail would not be likely to be lost. Pocock, who based this opinion on grounds

segment. Recently Tarnani (:04, text-figs. on pp. 51, 52, and 121) and Börner (:02, :04) have also adopted this view.

which are totally different from mine, accordingly divides the Arachnida into two sub-classes, viz. (1) Ctenophora¹ for the scorpions, and (2) Lipoctena for the remaining terrestrial orders. Börner (:02, p. 459) in his paper on the segmentation and general classification of the Arachnida, accepts this subdivision, but on other grounds, viz., on account of the difference in the number of the segments of the meso- and metasoma which appear to exist between the Scorpiones and the Lipoctena. Börner, however, considers that both Scorpiones and Lipoctena must have been derived from a common ancestral group provided with at least five pairs of lung-books (pp. 459 and 463), but the difficulty (in my opinion almost an impossibility) of deriving a lung-book from a pectine, or vice versâ, does not seem to have occurred to him.

The question of the conversion of a sunken-in lung-book into the external spinners of the Araneæ would also present difficulties, but these do not appear to me nearly so great as in the case of the pectines, because the reconversion of the lung-septa (lamellæ) into external gill-like organs is not involved. I have, however, already pointed out that no trace of a lateral proliferation, corresponding to that of the pulmonary and tracheal sacs, is found in the embryo of *Attus floricola*, the entire post-appendicular invagination becoming the entapophysis in these two segments. Moreover, the spinning glands appear at quite an early stage (stage 5, *sp. g.*, fig. 6) at the apex of the appendages, which always remain recognisable as such to the end of the development. In fact, they have every appearance of having been directly developed into spinning organs from external appendages which were not sunken into the body, and, therefore, not lung-books.

So far as our knowledge goes, therefore, we may say that there is no evidence of any sort to indicate that the spinners of the Araneæ were derived from

¹ For which word the term Cteidophora has been substituted by Börner (:02, p. 465).

sunken-in lung-books, or that the spinner-segments ever possessed such organs in any ancestral form of this order.

When abdominal segments bearing spiracles in other Tracheate orders (Solifugæ, Pseudoscorpiones, Opiliones, and Acari) are homologised with those bearing spinners in Araneæ, as is done by Börner (:02, p. 457), the difficulty of deriving spinners from lung-books should be taken into account. For if the *Lipoctena* represent a natural group and the tracheæ leading from these spiracles are derived from lung-books, as is often assumed to be the case, it follows that the spinners in Araneæ must also have been derived from lung-books. But if we cannot admit the latter derivation, then either some or all of these tracheæ are not homologous with lung-books (i. e. they are new formations), or else the segments bearing them are homologous with the pulmonary segments in Araneæ (and not with those bearing spinners), or, finally, some or all of these orders may have originated independently of the Pedipalpi and Araneæ from branchiate ancestors (whether in connection with the Scorpiones or not is another question).

In the Solifugæ two (or at least one) of the three tracheate segments of the abdomen must be homologous with segments bearing spinners in Araneæ, and a knowledge of the development of the tracheæ would be necessary before one could determine the relationships of this order.

(4) *Limulus*.—According to Kingsley ('85) the genital segment in the American species of *Limulus* is the seventh post-oral segment, but Kishinouye ('91) has since discovered an additional somite between the last thoracic segment and the genital segment in the Japanese species, thus making the latter segment the eighth post-oral one. It is possible that this additional seventh somite was overlooked in the American *Limulus*, just as it has frequently been overlooked in the spiders and scorpions, for its presence would bring the segmentation of the abdomen of *Limulus* into line with that of spiders and scorpions.

Thus the homologies of the abdominal appendages in *Limulus* may with the greatest probability be represented as in the table on p. 86.

XI. HISTORICAL LIST OF PAPERS CONCERNING THE LUNG-BOOKS OF ARACHNIDS (EXCLUSIVE OF THOSE DEALING ONLY WITH ENERVATION, EMBRYOLOGY OR THEORETICAL CONSIDERATIONS.¹)

Meckel ('09 and '10) gives the earliest anatomical description of the lung-books of a scorpion and spider. He observed the leaflets (saccules) attached to a stalk (pedicel and ante-chamber) leading to the spiracle, and thought the stalk might be hollow. He looked upon the organ as a real gill-book.

G. R. Treviranus ('12, '16) describes the lung-books of scorpions and spiders (as true gills) and the interpulmonary fold and its muscles in spiders. He thought the "gills" may be mainly organs for absorbing moisture from the air, while the respiratory functions are carried out principally by four pairs of stigmata on the back of the abdomen (muscle impressions) and four pairs on the sides of the cephalothorax.

H. M. Gaede ('23) describes the four lung-books of a Mygale (as gills) and observed the "granulation" on the leaves. He thought the respiration took place, not in the "gill-leaves," but on the fine membrane behind them (i. e. on the posterior wall of the ante-chamber, which is smooth in some Tetrapneumonous spiders, e. g. in *Crypsidromus intermedius*).

Johannes Müller ('28A, '28B) discovered that the stalk (pedicel), ante-chamber and leaves (saccules) in scorpions and spiders are hollow by blowing air into the spiracle, and so proved the pulmonary function of the lung-books. He

¹ The comparative anatomy of the lung-books is outside of the scope of this paper, so I give this list in the form of an appendix for the use of future workers on the subject, as it is more complete than any list yet given.

correctly surmises the passage of the blood between the saccules and denies the presence of blood-vessels. This is the most important description up to Leuckart's time.

H. Straus-Durckheim ('28) describes the lung-books of spiders, and says one may consider the saccules of these organs in Arachnids as non-ramified tracheal trunks, representing merely a continuation of the external integument in-folded into the interior of the spiracles (pp. 315-318).

J. F. Brandt ('33, p. 89) gives a poor description of the lung-books of *Epeira diadema* (as gills), apparently without knowledge of the work of the two previous authors.

A. Dugès ('36, p. 181) injected spiders' lung-books with carmine. There is also a note on the lung-books in Dugès, '38, p. 568, teste Duvenoy ('40, p. 465).

G. L. Duvenoy ('40) describes the lung-books of spiders.

J. van der Hoeven ('42) describes the lung-books of *Phyrnus medius*,¹ calling them gills.

G. Newport ('43) describes the appearance of the lamellæ in scorpions and the circulation of the blood through the lung-books ("branchiæ") (pp. 295-297).

Pappenheim ('48) has a note on the lung-books of spiders.

A. Dugès ('49) gives figures of the lung-books of *Mygale* (Pl. ii, fig. 8, and Pl. iv, fig. 6), *Segestria* (Pl. iv, fig. 5), *Pholcus* (Pl. iv, fig. 7), and *Scorpio* (Pl. xviii, fig. 1*f*).

R. Leuckart ('49) describes the lung-books of scorpions and spiders. He discovered the spines of the ante-chamber in spiders and recognised the network on the leaves in scorpions as a chitinous thread on the surface of the membrane. He insists that lung-books are merely modifications of tracheæ (also '48, p. 119 note), and his paper is the most important that appeared before MacLeod's.

E. Blanchard ('49, '50) proved by injection that the blood passes through the septa and thence to the heart.

A. Menge ('51) describes the lung-books of *Argyroneta* (water spider). He failed to find any respiratory movements

¹ According to Kraepelin ('95, p. 41) v. d. Hoeven's species was in reality *Charon Grayi*, Gerv.

either in the lung-books or in the enclosed air, and observed that the entire cuticula of these organs is shed at moulting. He doubted their respiratory function.

F. Leydig ('55) found that the "granulations" observed by previous authors in the lung-leaves of spiders are really internal processes, like those in the posterior tracheæ.

L. Dufour ('56) describes the scorpion's lung-books (pigment and reticulation of the leaves, etc.).

E. Claparède ('63) describes the circulation of the blood, with some notes on the lung-leaves, in the spider.

P. Bertkau ('72) gives a good description of the lung-books in spiders, and the earliest account of their growth in young spiders.

C. Chun ('76), from a brief remark (p. 42), evidently implies that he has found an epithelium with regular cell-boundaries on the lung-leaves of Arachnids, but reserves the proof for a later occasion.

H. Lebert ('77, p. 25) makes some very curious observations, such as his discovery of a second pair of smaller lung-books (*Nebenfächertracheen*) in other spiders besides *Tetraneumon*es (e. g. in some *Argiopidae*); also bifurcate sacculi.

J. MacLeod ('80, '82, '84) advanced our knowledge greatly beyond the works of his predecessors by the use of sections. In his first paper ('80) he describes the lung-books as "*un faisceau de trachées aplatis, foliiformes*" (p. 48), but influenced later by the branchial theory he re-casts his method of treating the subject ('82, '84). His principal paper ('84) is, perhaps, the best known of all works on the lung-books.

E. Ray Lankester ('81, '85a, '85b) in his first paper compares the lung-books of scorpions with the gill-books of *Limulus* from actual preparations, and derives lung-books from gill-books by a theory. This paper ('81) affected most subsequent studies of the subject, and made the development and comparative anatomy of the respiratory organs a subject of paramount interest in the Arachnida. His later papers ('85a, '85b) describe the circulation of the blood through the

lung-books, and the histology of the lamellæ in *Scorpio*, and a new theory of the origin of the lung-books.

P. J. Mitrofanof ('81) makes some remarks on the lamellæ in *Argyroneta* (teste Schimkewitsch '84, p. 64).

W. Schimkewitsch ('84) describes the lung-books of *Epeira*, without knowledge of MacLeod's principal paper ('84).

F. Plateau ('86) searched for respiratory movements in living *Scorpiones*, *Araneæ*, and *Opiliones*, with negative results.

W. Wagner ('88) describes the moulting and growth of the lung-books in immature spiders.

L. Berteaux ('89) describes minutely the lung-books of spiders and scorpions. This paper is the most complete on the histological structure, particularly that of the cuticular formations, and various errors made by MacLeod ('84) in this respect are corrected. (His description of the form of the lung-leaves and the ante-chamber is, however, unsatisfactory, and is improved upon by Schneider ['92].)

J. Tarnani ('89) figures the topography of the two pairs of lung-books in *Thelyphonus*, and describes the interpulmonary folds. In his later work (:04) these lung-books are also figured and described (p. 121).

C. Vogt ('89) gives an original description of the lung-books of *Epeira diadema*.

A. Schneider ('92) describes the circulation of the blood through the lung-books, and gives an account of the general structure of these latter organs in spiders. This very excellent paper is indispensable as a supplement to Berteaux's important histological work.

M. Laurie gives an account of the structure and histology of the lung-books in *Pedipalpi* ('94), and of the difference in the chitinous armature of the septa in different groups of scorpions ('96a, 96b).

Sophie Pereyaslawzewa (:01) figures some sections of the lung-books of *Phrynidæ* (figs. 59, 62 and 64), and gives a number of descriptive notes, especially on the histology of the septa and on the pulmonary muscles (pp. 251-262).

A. Börner (:04) gives an account of the lung-books in Pedipalpi, and some diagrams to illustrate their structure in Arachnids generally.

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EXPLANATION OF PLATES 1—7,

Illustrating Mr. W. F. Purcell's paper on "Development and Origin of the Respiratory Organs in Araneæ."

STAGES IN THE DEVELOPMENT.

Stage 1 (*St. 1*), just before the appearance of the pulmonary furrows : figs. 4, 7, and 7A (all from same embryo).

Stage 2 (*St. 2*), with two pulmonary furrows : figs. 1, 5, 5A, 8, and 8A-8H (5, 5A, 8, 8A-8H all from one embryo) ; figs. 9, 10, 14.

Stage 3 (*St. 3*), with three pulmonary furrows : figs. 2, 11.

Stage 4 (*St. 4*), with 4-5 pulmonary furrows : figs. 12, 15.

Stage 5 (*St. 5*), with 5-6 pulmonary furrows : figs. 3, 6, 6A, and 27 (6, 6A, and 27 from one embryo) ; figs. 13, 13A, and 13B (all from one embryo) ; figs. 16, 16A-16E, 35, and 35A (all from one embryo).

Stage 6 (*St. 6*), after end of reversion and shortly before hatching : figs. 17 and 43 (from same embryo) ; figs. 18, 28, 41.

Stage 7 (*St. 7*), after hatching : fig. 34.

Stage 8 (*St. 8*), after first post-embryonic moult : figs. 29 and 29A (from same embryo) ; fig. 30.

ABBREVIATIONS FOR ALL THE PLATES.

The yolk is coloured yellow in the figures representing sections, all of which have been drawn with the aid of a drawing apparatus. The letters (*St. 1*), (*St. 2*), etc., alongside the numbers of the figures denote the stage of the embryo from which the section has been made.

a. ob. m. 8 and 10. Anterior oblique muscles of somites 8 and 10.
a. spin. Inner openings of anterior pair of spinners. *ab. app.* 1-4. Abdominal appendages 1-4. *ant.* Anterior side. *ap.* Apical pouch of horn of pulmonary sac in developed lung-book. *app.* 6. Sixth prosomatic appendage. *ar.* 7-11. Areas in contact with the ends of the segments of the ventral longitudinal muscles of somites 7-11 at the time of the formation of the entochondrites. *b.f.* Basal fold of tracheal trunk. *bd. c.* Blood-corpuscles. *br.* Branches of tracheal trunk. *c.* Cones of a chitinous saccule. *can.* Canal of communication. *cent.* Centre of section. *cl. 1, cl. 2.* Clefts on the distal side of first and second pulmonary saccules. *cœl.* 6-14. Cœlomic sacs of 6th-14th post-oral somites. *cu.* Cuticula. *cu', cu''* Cuticula formed at first and second post-embryonic moults. *d. l. m.* 8-15. Segments of the dorsal longitudinal muscles in somites 8-15. *d. v. l. m.* Longitudinal muscle alongside of the proctodæum uniting the last segment of the dorsal with that

of the ventral longitudinal muscles. *d. v. m.* 7-10. Dorso-ventral muscles behind somites 7-10. *dist.* Distal side. *dors.* Dorsal side. *dors. (lat.)*. Dorsal (originally lateral) side. *ec. g. d.* ectodermal portion of genital duct. *ec. t.* 8-11. Ectodermal tendons (entapophyses, apodemes) of the appendages of the 8th-11th somites. *end.* Endoderm. *ep.* epithelium. *f. 1, f. 2*, etc. First, second pulmonary furrows, etc., in the order of their formation. *g.* Genital cord. *g. o.* Genital opening into interpulmonary fold (to the outside in fig. 40). *gr.* Groove behind abdominal appendages. *h.* Horn (procurved end) of pulmonary sac. *horiz. pl.* Horizontal plane of body. *hy.* Hypodermis. *hy'*. Fibrous parts of the hypodermis of the ectodermal tendons. *inf.* Infolding of the hypodermis. *interp. fld.* Interpulmonary (epigastrie) fold or its rudiment. *l.* Lumen. *l. spin. m.* 10. Lateral muscle to anterior side of first spinner (in 10th somite). *l. tr.* Lateral trunks of tracheal system. *lac.* Lacuna. *lat.* Lateral side. *lb.* Lung-book or tissue forming it. *m.* Muscles or tissue forming them. *m. spin.* Inner openings of middle pair of spinners. *m. spin. m.* 10. Medial muscle to anterior side of first spinner (in 10th somite). *m. tr.* Medial trunks of tracheal system. *ma.* Matrix cells. *med.* Medial side. *med. pl.* Median plane. *mes. g. d.* Mesodermal part of genital duct. *nv. g.* Nervous ganglion of the pulmonary somite. *op.* Opening at anterior end of abdomen. *p. ob. m.* 8-11. Posterior oblique muscles of somites 8-11. *p. spin.* Inner openings of posterior pair of spinners. *ped.* Pedicel. *post.* Posterior side. *pr. ax.* Principal axis of appendage. *proc.* Proctodæum. *pulm. l.* Lumen of pulmonary sac. *pulm. prol.* Pulmonary proliferation or growing end of pulmonary sac. *pulm. s.* Pulmonary sac. *rd.* Chitinous thickening on lateral side of tracheal pedicel. *s., s. 1, s. 2*, etc. Pulmonary sacculi; first, second sacculi, etc., in the order of their formation. *s'*. New chitinous sacculi forming before the first post-embryonic moult. *seg. t.* 8-11. Segmental tubes of 8th-11th somites. *sl.* Slanting medial part at base of posterior wall of first appendage. *sp.* Spiracle. *sp. g.* Spinning gland. *spi.* Anastomosing spines. *spin. m.* 10. Muscles to the anterior side of first pair of spinners. *spz.* Sperma. *st. p.* Stercoral pocket. *t.* Mesodermal tendon (entochondrite). *t.* 7-11. Entochondrites at hind ends of the segments of the ventral longitudinal muscles of somites 7-11. (*t.* 8-11.) Indicates the position of these tendons, where not drawn in. *tr.* Tracheal trunk. *tr. l.* Lumen of tracheal plate or sac. *tr. m.* Transverse muscle on lateral side of tracheal pedicel. *tr. pl.* Tracheal plate. *tr. prol.* Tracheal proliferation. *tr. s.* Tracheal sac. *tr. tub.* Tracheal tubules or finest branchlets. *v. l. m.* 7-11. Segments of the ventral longitudinal muscles in somites 7-11. *v. sin.* Ventral sinus of abdomen. *ve.* Vacuole. *vent.* Ventral side. *vent. (med.)*. Ventral (originally medial) side. *vest.* Vestibule. *vit.* Vitellophagous cell. *w.* Two-celled column. *x.*

Point at which the entochondrite is attached to the hypodermis. *y*. Three-celled column. *z*. Pavement cell. *w*. *x'*, *x'*. *y'*, and *y'*. *z'*. Anterior, distal, and posterior sides of appendage.

PLATE 1.

Embryology of *Attus floricola*.

[Longitudinal sections are cut parallel to the principal axis (*pr. ax.* in figs. 1-3) of the appendage and at right angles to the posterior margin of the latter. Transverse sections are parallel to the principal axis and to the posterior margin.]

Figs. 1-3.—(Zeiss obj. C, oc. I, hot alc. subl.) Transverse sections showing the change of position of the first pair of abdominal appendages during the reversion of the embryo. Fig. 1 represents the stage with two pulmonary furrows, fig. 2 with three, and fig. 3 with four, five, or more furrows.

Fig. 4.—(Zeiss C, III, hot alc. subl.) Longitudinal section through the lateral region of the four abdominal appendages, just previous to the appearance of the pulmonary furrows and the commencement of the reversion.

Figs. 5 and 5A.—(Zeiss C, III, hot alc. subl.) Longitudinal sections through the abdominal appendages at the stage with two pulmonary furrows (*f. 1, f. 2*), corresponding to fig. 1. Fig. 5 passes through the medial, fig. 5A through the lateral region of the anterior appendages.

Figs. 6 and 6A.—(Zeiss C, III, hot alc. subl.) Longitudinal sections through the abdominal appendages at the stage with five or six pulmonary furrows, corresponding to fig. 3. Fig. 6 passes through the medial, fig. 6A through the lateral region of the appendages.

Figs. 7 and 7A.—(Zeiss $\frac{1}{2}$ oil im., II, hot alc. subl.) Longitudinal sections through the medial (fig. 7) and lateral part (fig. 7A) of the first abdominal appendage (enlarged from the same embryo as fig. 4) just before the formation of the pulmonary furrows; *ep.*, epithelium (belonging to somite 9) behind first appendage.

PLATE 2.

Embryology of *Attus floricola*.

[Longitudinal sections are cut parallel to the principal axis (*pr. ax.* in fig. 1) of the appendage.]

Fig. 8.—Diagrammatic view of the posterior side of the first abdominal appendage at the end of the stage (corresponding to fig. 1 with two pulmonary furrows, *f. 1, f. 2*) (from a wax reconstruction). The parallel lines represent the planes of sections; *ep.*, the epithelium

represented as cut along the groove (*gr.*) in figs. 8A-8D, and along the deepest part of the pulmonary sac (*pulm. s.*) in figs. 8F and 8G.

Figs. 8A-8H.—(Zeiss $\frac{1}{12}$ oil im., II, hot alc. subl.) Longitudinal sections through the first abdominal appendage, of which fig. 8 is a reconstruction. Figs. 8A-8E pass through the medial and 8F-8H through the lateral halves of the appendage, and their positions are indicated in fig. 8; *ep.*, epithelium (belonging to somite 9) behind the first abdominal appendage.

PLATE 3.

Embryology of *Attus floricola*.

[Longitudinal sections are cut parallel to the principal axis (*pr. ax.* in figs. 1-3) of the appendage.]

Figs. 9 and 10.—(Zeiss $\frac{1}{12}$ oil im., II, hot. alc. subl.) Longitudinal sections (from different embryos) through the medial region of the first abdominal appendage at the commencement (fig. 9) and the end (fig. 10) of the stage with two pulmonary furrows; *ep.*, epithelium (belonging to somite 9) behind first appendage.

Fig. 11.—(Zeiss $\frac{1}{12}$ oil. im., II, hot alc. subl.) Section through the medial region of the first abdominal appendage at about the commencement of the 3-furrow stage, cut at a slight inclination to the longitudinal axis of the appendage.

Fig. 12.—(Zeiss $\frac{1}{12}$ oil im., II, hot alc. subl.) Longitudinal section through the lateral region of the first abdominal appendage at the stage with four to five pulmonary furrows.

Figs. 13-13B.—(Zeiss $\frac{1}{12}$ oil im., II, hot alc. subl.) Transverse sections cut parallel to the anterior side of the first abdominal appendage at a stage with at least five well-developed pulmonary furrows, fig. 13 being the second, 13A the fifth, and 13B the eighth section from the posterior side of the appendage (13 and 13A are in outline and show ectodermal tissue only). *sp.* The primitive spiracle; *s.* 1-s. 5, the five oldest saccules.

Fig. 14.—Sketch of first abdominal appendage of the right side at the stage with two pulmonary furrows, from a wax reconstruction, seen from behind and distally.

Fig. 15.—(Zeiss $\frac{1}{12}$ oil im., II, hot alc. subl.) Sagittal section (cut parallel to the median plane of the embryo, c.f. fig. 2) through the first abdominal appendage at the commencement of the stage with four pulmonary furrows (slightly later than fig. 2).

PLATE 4.

Embryology of *Attus floricola*.

[Longitudinal sections are cut parallel to the principal axis (*pr. ax.* in fig. 3) of the appendage.]

FIG. 16.—Diagrammatic view of the posterior side of first abdominal appendage at the stage with five well-developed pulmonary furrows (corresponding to fig. 3) from a wax reconstruction. The parallel lines represent the planes of section; *ep.*, the epithelium represented as cut along the line marked (*ep.*, fig. 16) in figs. 16A–16D.

FIGS. 16A–16E.—(Zeiss $\frac{1}{12}$ oil im., II, hot alc. subl.) Longitudinal sections through the first abdominal appendage, of which fig. 16 is a reconstruction.

FIG. 17.—(Zeiss $\frac{1}{12}$ oil im., II, hot alc. subl.) Transverse section through first abdominal appendage shortly before the hatching of the embryo, cut along the line indicated in fig. 18.

FIG. 18.—(Zeiss $\frac{1}{12}$ oil im., II, hot alc. subl.) Sagittal section of the same stage as fig. 17 and cut along the line indicated in the latter figure.

PLATE 5.

FIG. 19.—(Zeiss $\frac{1}{12}$ oil im., I.) Sagittal sections through the cuticula of the lateral part of the interpulmonary fold (between the entapophysis and the lung-book), or its rudimentary remains in A, *Lycosa* sp.; B, *Philodromus fuscumarginatus* (subadult); and C, *Argyroneta aquatica* (adult ♂). The sections are all arranged in the same positions.

FIG. 20.—(Caustic potash.) *Attus* sp. (adult). Chitinous skeleton of abdomen anterior to the pulmonary spiracles, drawn from behind. The posterior wall of the ante-chamber has been removed on the right side, and on the left the grate-like openings are visible through this wall.

FIG. 21.—(Caustic potash.) *Tegenaria domestica* (ad. ♀). Chitinous skeleton of abdomen from above.

FIG. 22.—(Zeiss $\frac{1}{12}$ oil im., I, picro-sulphuric acid.) *Attus floricola* (mature, or nearly mature ♀). Sagittal section through the interpulmonary fold between the lung-book and the entapophysis, showing the cuticula only.

FIG. 23.—(Zeiss $\frac{1}{12}$ oil im., I, Flemming's sol.) *Attus floricola* (ad. ♂). Sagittal section through the interpulmonary fold and the entapophysis in the region indicated in fig. 20.

Figs. 23A and 23B.—Similar sections of the same ♂ in the regions indicated in fig. 20. Fig. 22A shows the cuticula only.

Fig. 24.—(Zeiss $\frac{1}{2}$ oil im., IV.) *Linyphia triangularis* (ad. ♂). Sagittal section through the vestibule of the trachea in the region indicated in fig. 25, showing the cuticula only.

Fig. 25.—(Zeiss C, IV, caustic potash.) *Linyphia triangularis* (ad. ♀). Cuticular skeleton of basal region of tracheal system.

Fig. 26.—The same as fig. 24, but in the median plane along the line indicated in fig. 25.

PLATE 6.

Adult or sub-adult spiders.

Fig. 27.—(Zeiss F, I, hot alc. subl.) Embryo of *Attus floricola* at the stage with 5-6 pulmonary furrows. Reconstruction (made from the same series of sections as figs. 6 and 6A) of the right pulmonary and tracheal appendages seen from their inner side. The only mesodermal elements shown are the two segmental tubes, *seg. t.* 8 and 9. The basal outlines of appendages 1-3 are indicated by dotted lines, as are also the lumens of the pulmonary and tracheal sacs.

Fig. 28.—(Zeiss F, I, hot aqueous subl.) Embryo of *Attus floricola* after the reversion. Reconstruction from transverse sections of the rudimentary trachea, together with the muscles and entochondrites attached to the right half. The sketch is imagined as taken directly from above, the anterior part being therefore higher in the figure than the posterior part (cf. also figs. 41 and 43 of the same stage). The rudimentary lumen is outlined by the dotted line.

Fig. 29.—(Zeiss F, I, hot alc. subl.) Young *Attus floricola*, after the first post-embryonic moult. Reconstruction of the tracheal system (imagined as taken directly from above) together with the muscles and entochondrites connected with the right half and some of those on the left. 29A (Zeiss $\frac{1}{2}$ oil im., IV). Transverse section through a right medial trunk at the line indicated in fig. 29.

Fig. 30.—(Zeiss $\frac{1}{2}$ oil im., II, hot alc. subl.) Young *Agelena labyrinthica* just before the second post-embryonic moult. Sagittal section through a lateral tracheal trunk and the hypodermis below it; the cuticula of the second moult is already formed.

Fig. 31.—(Zeiss C, III, caustic potash.) *Attus floricola* (ad. ♀). Tracheal system. (The terminal portions of the secondary tubules are not drawn in.)

Fig. 32.—(Zeiss C, III, equal parts of Flemming's sol. and abs. alc.)

Segestria senoculata (ad. ♀). Obliquely transverse section cut at an angle of 38° to the horizontal (cf. fig. 33) though the basal part of a tracheal trunk (combined from a couple of sections).

Fig. 33.—Similar to the last but a sagittal section, showing the entapophysis cut across the line indicated in fig. 32.

Fig. 34.—(Zeiss $\frac{1}{2}$ oil im., IV, Flemming's sol.) Embryo of *Tegenaria atrica*, just after hatching. Transverse section through two upper pulmonary saccules.

PLATE 7.

Figs. 35 and 35A.—(Zeiss $\frac{1}{2}$ oil im., II, hot alc. subl.) Embryo of *Attus floricola* at the stage with five pulmonary furrows (from the same series of sections as figs. 16–16E). Longitudinal sections through the tracheal appendage along the lines indicated in fig. 27.

Fig. 36.—(Zeiss $\frac{1}{2}$ oil im., I.) *Crypsidromus intermedius*. Sagittal section through the rudimentary entapophysis or muscular stigma of the first pulmonary sternite, showing the distal part of some of the long hypodermal fibres (*hy.*¹) to which the entochondrite of the pulmonary segment is attached.

Figs. 37 and 37A.—(Zeiss $\frac{1}{2}$ oil im., I, alc.) *Palpimanus* sp. Transverse sections through the anterior (fig. 37) and the posterior (fig. 37A) regions of the median entapophysis of the tracheal system.

Fig. 38.—(Zeiss C, IV, caustic potash.) *Scytodes testudo*. Basal part of chitinous skeleton of the tracheal system.

Fig. 39.—(Zeiss $\frac{1}{2}$ oil im., IV, alc.) *Scytodes testudo*. Transverse section through the cuticular lining of the median entapophysis of the tracheal system along the line indicated in fig. 38.

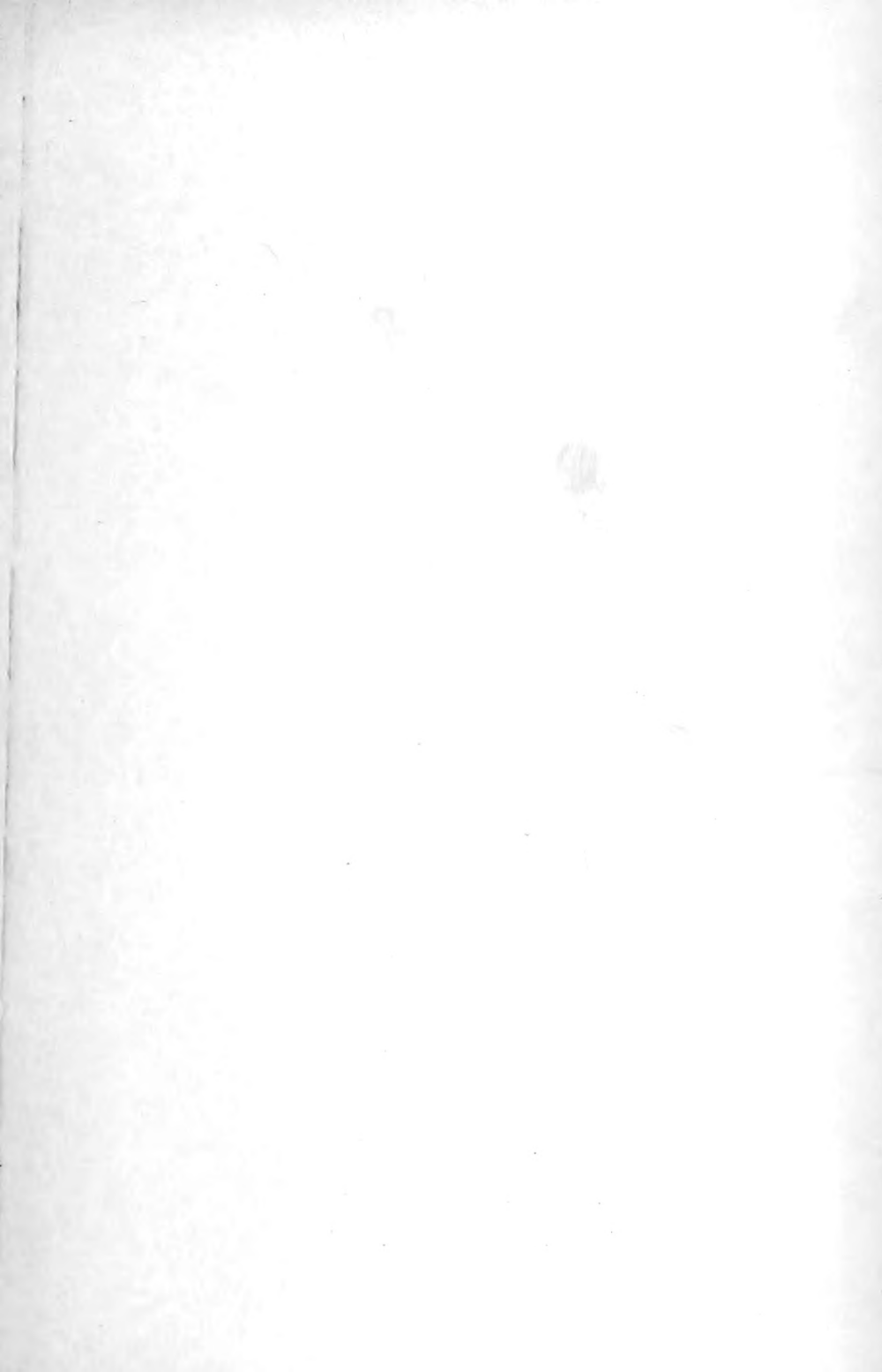
Fig. 40.—(Zeiss $\frac{1}{2}$ oil im., I.) *Harpactes Hombergi* (ad. ♂). Median sagittal section through the cuticula of the interpulmonary fold and the genital opening.

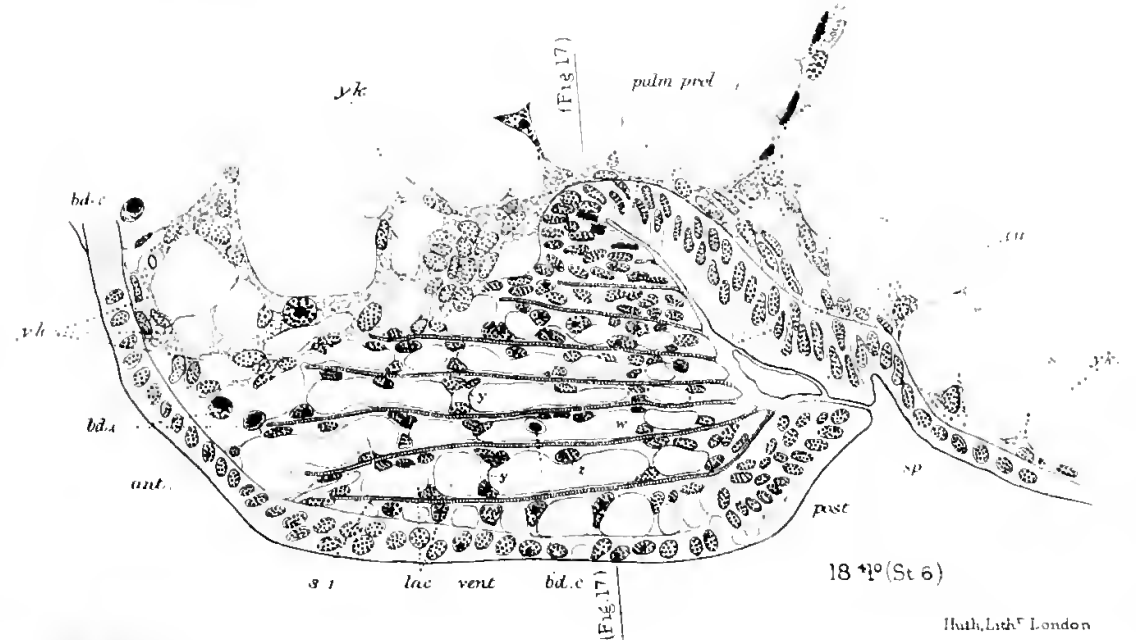
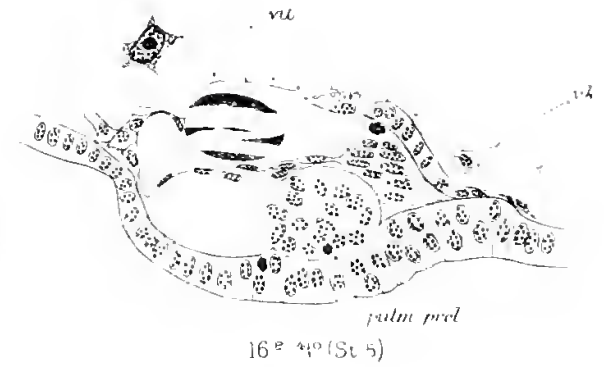
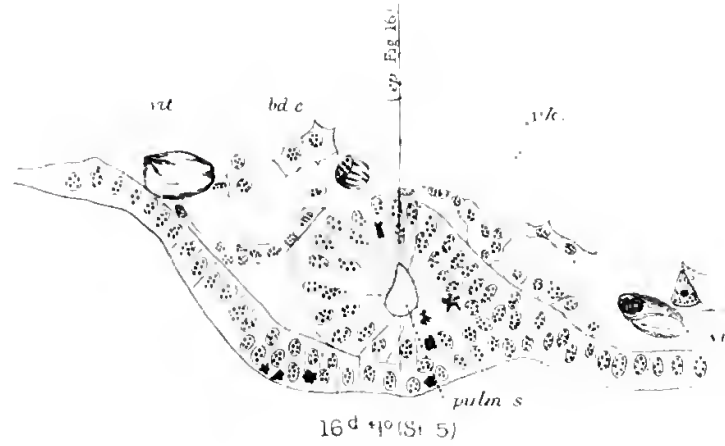
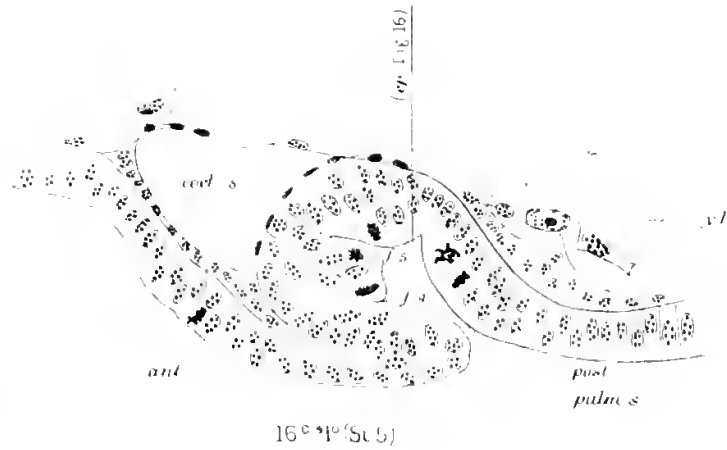
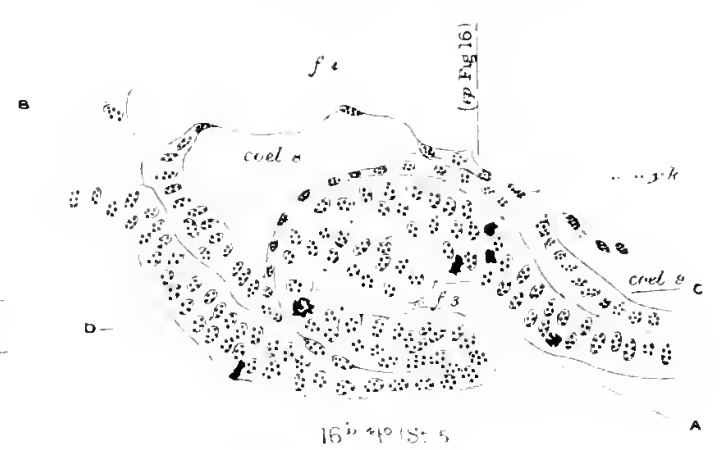
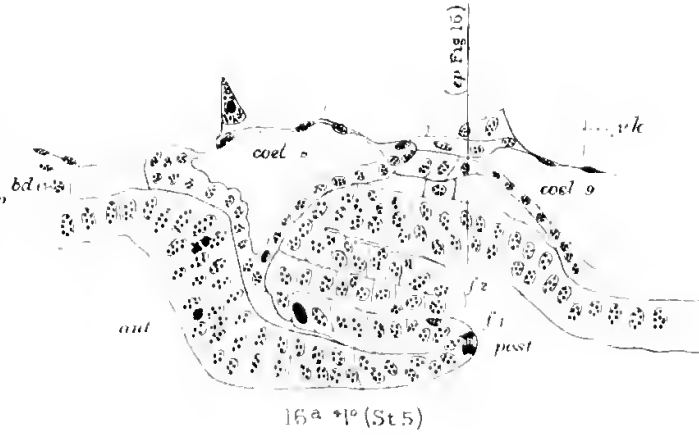
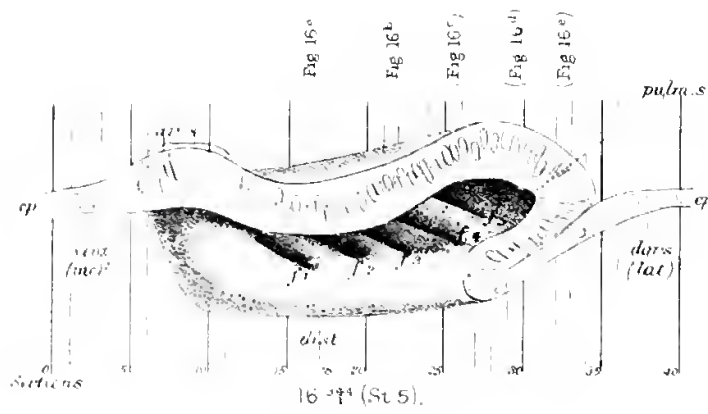
Fig. 41.—(Zeiss C, II, hot alc. subl.) Embryo of *Attus floricola* after the reversion in sagittal section, showing the principal muscles, ectodermal and mesodermal tendons and segmentation of the abdomen (combined from several sections). The lungs, genital cords, and the stercoral pocket (imagined as seen from the medial side) are drawn in to show their topography, the last being represented in median section. The muscle (*p. ob. m.* 8) is the only one lying between the genital duct (*g.*) and the lung-books (*lb.*).

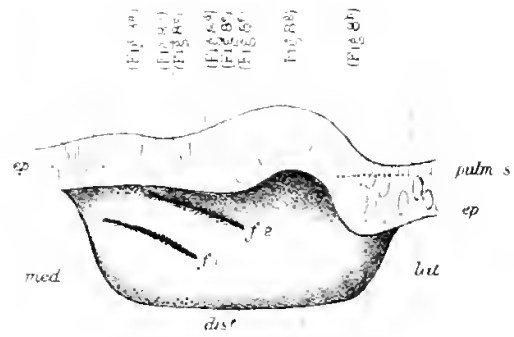
Fig. 42.—(Zeiss A, II, caustic potash.) *Tegenaria domestica* (adult). Portions of the pair of median tracheal trunks, showing the

place of attachment to the entochondrite (seen from the dorsal side). A, part of left trunk; B, anterior part of right trunk (see fig. 21).

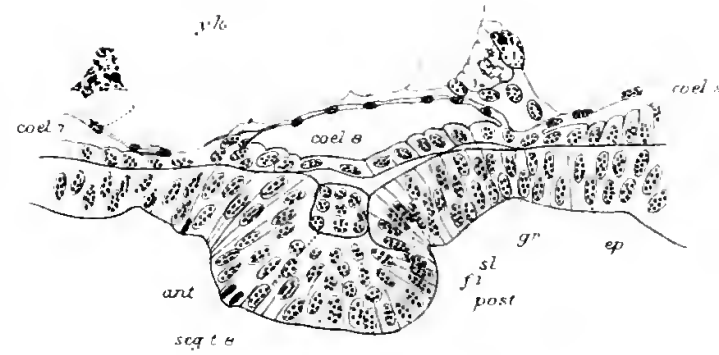
Fig. 43. — (Zeiss $\frac{1}{12}$ oil im., II, hot alc. subl.) Embryo of *Attus floricola* after the reversion. Transverse section through the ventral sinus of the abdomen in the region of the tracheal plate (along the line indicated in fig. 28; same stage as figs. 28 and 41).



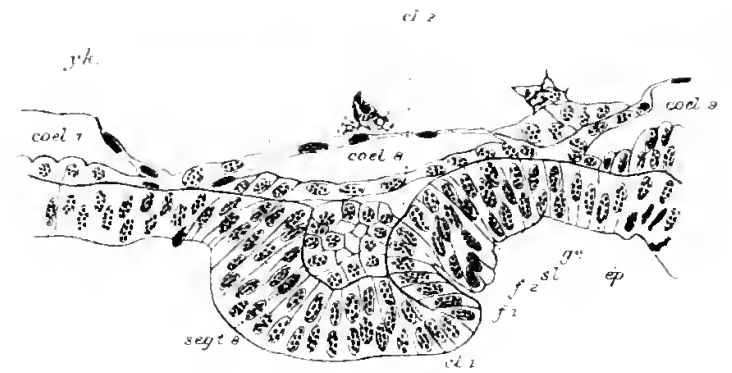




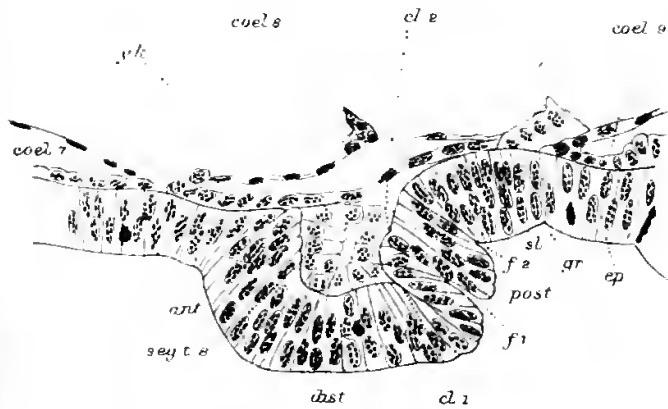
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8.b (St 2)



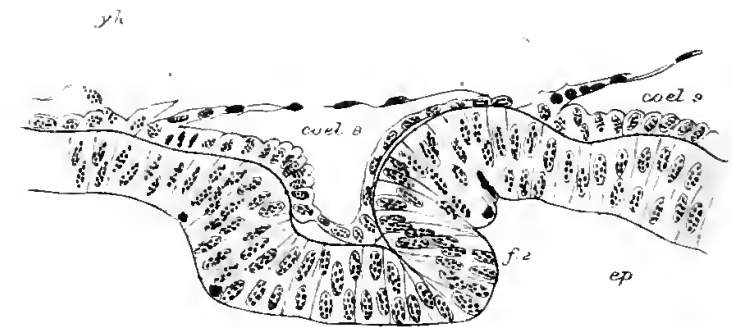
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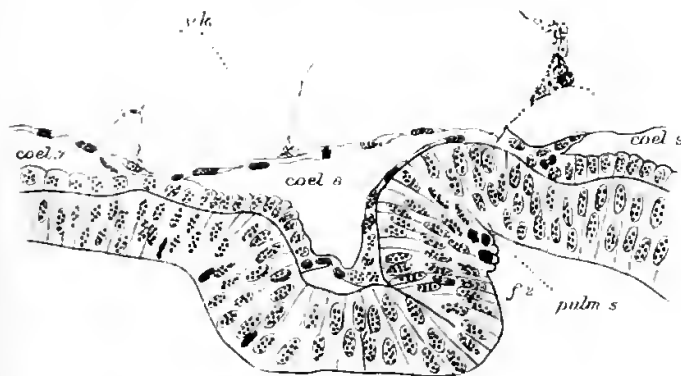
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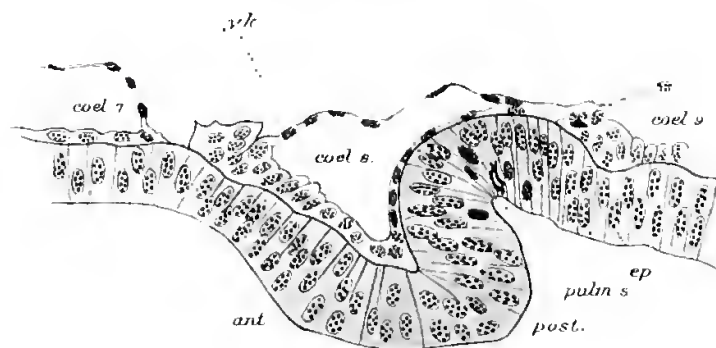
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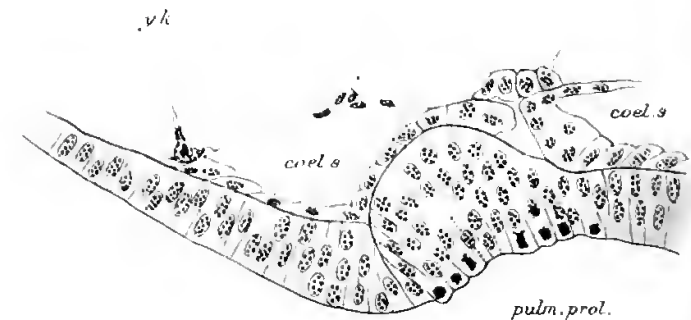
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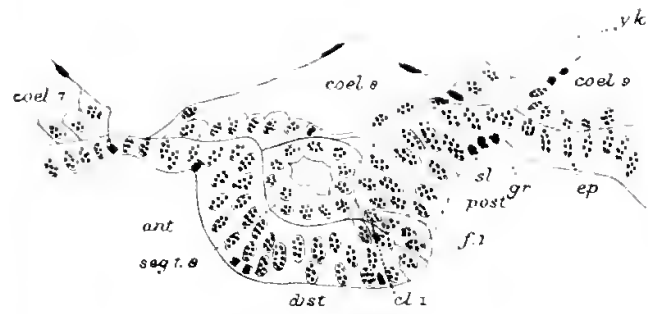
8.f (St 2)



8.g (St 2)



8.h (St 2)



9. ♀ (St 2)



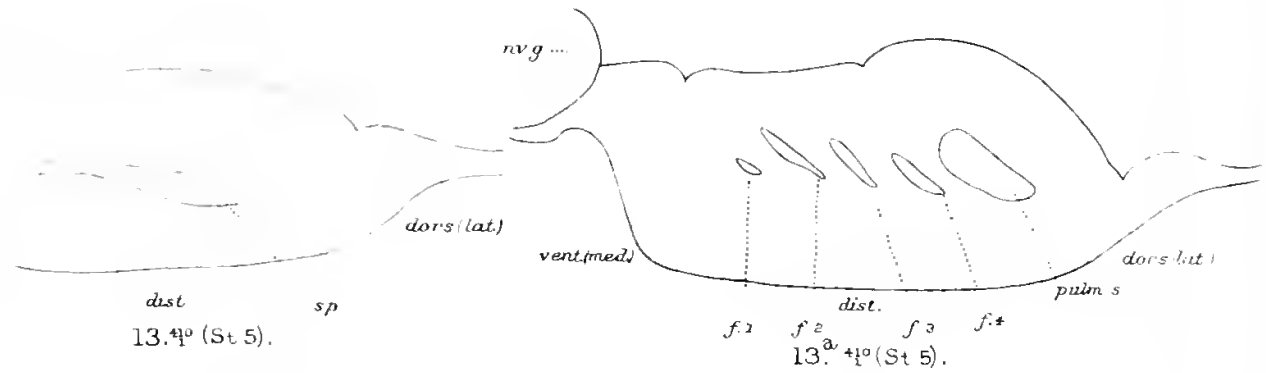
10. ♀ (St 2)



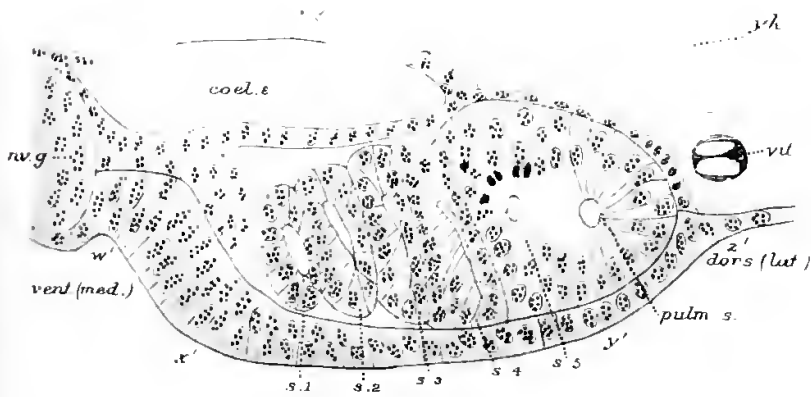
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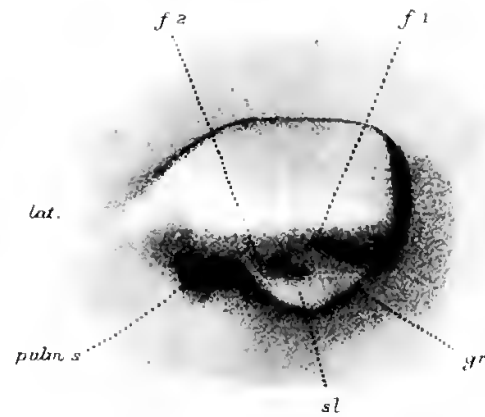
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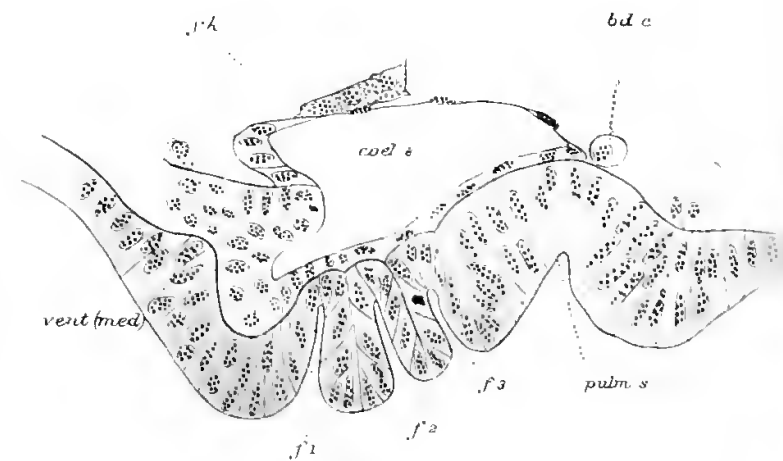
13. ♀ (St 5).



13. ♀ (St 5).

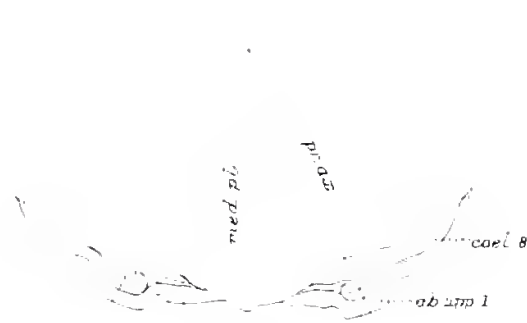


14. ♀ (St 2)

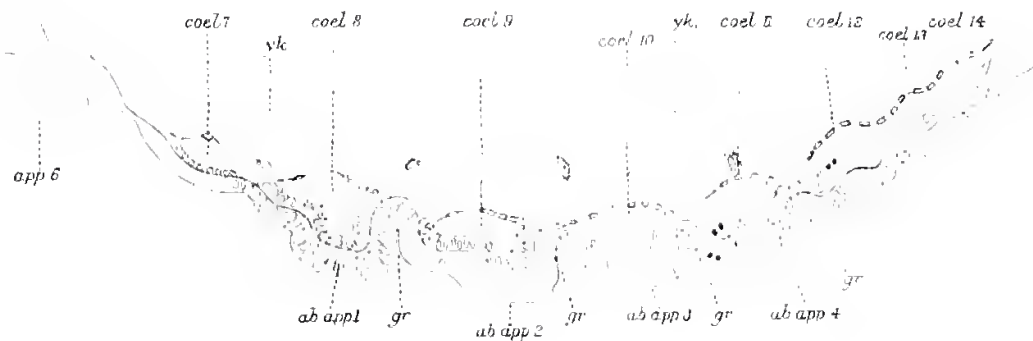


15. ♀ (St 4)





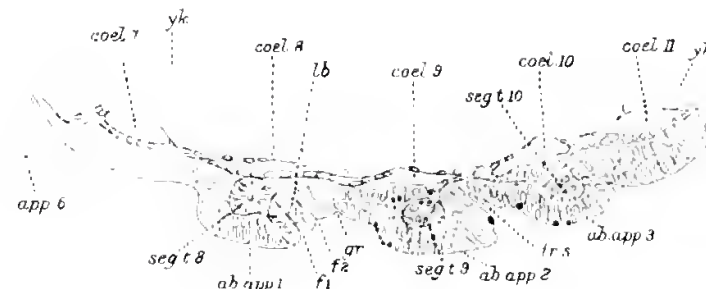
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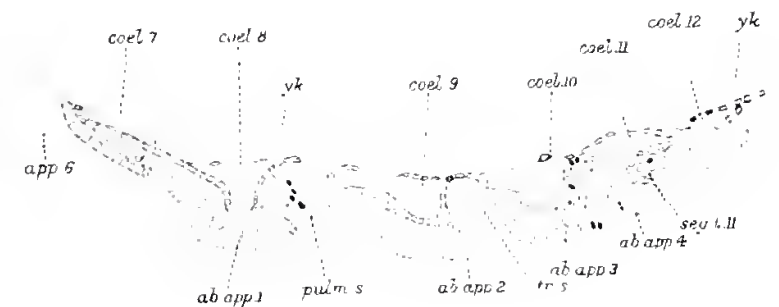
4. 1° (St. 1)



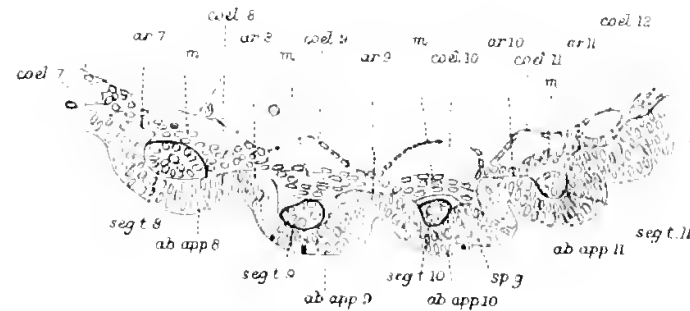
2. 2° (St. 3)



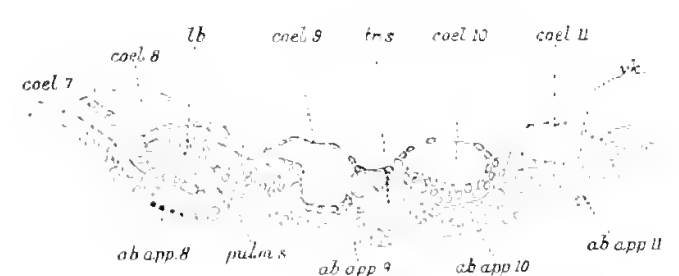
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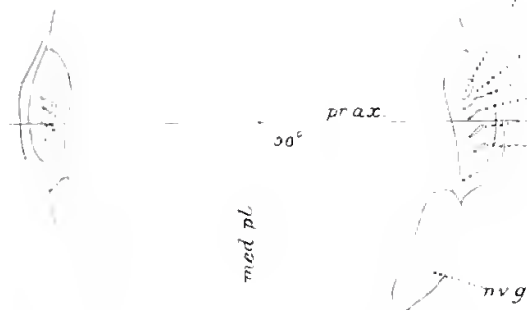
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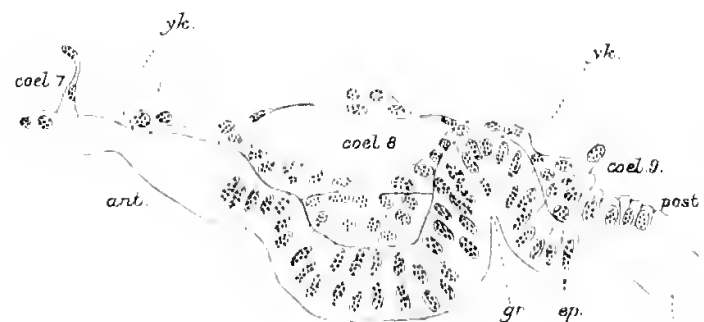
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6a. 1° (St. 5)



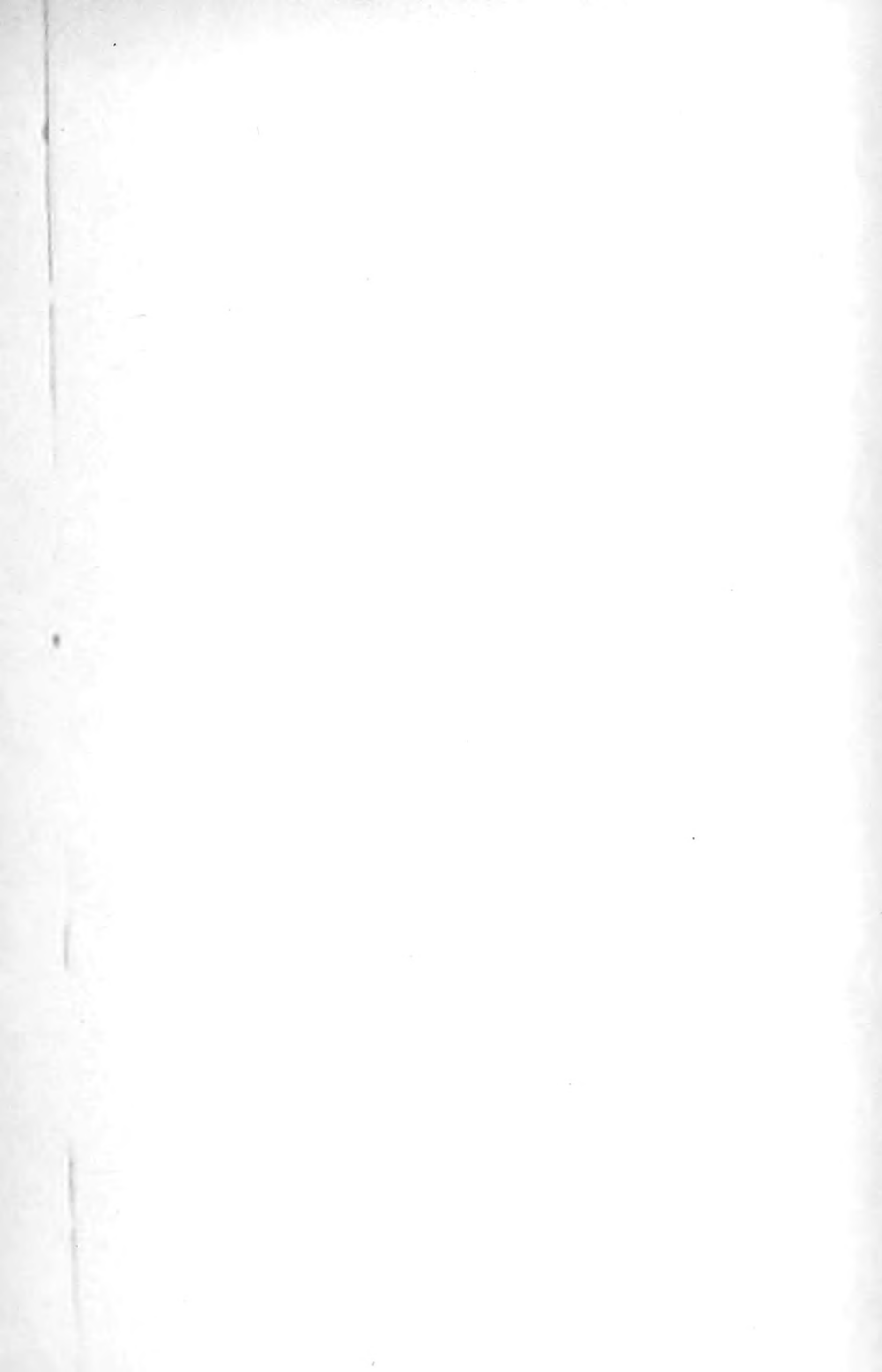
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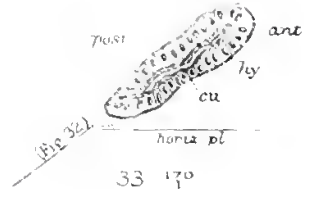
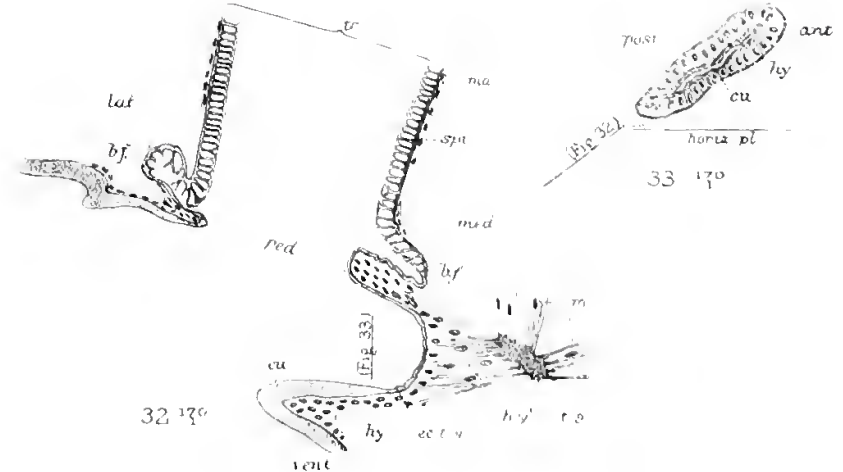
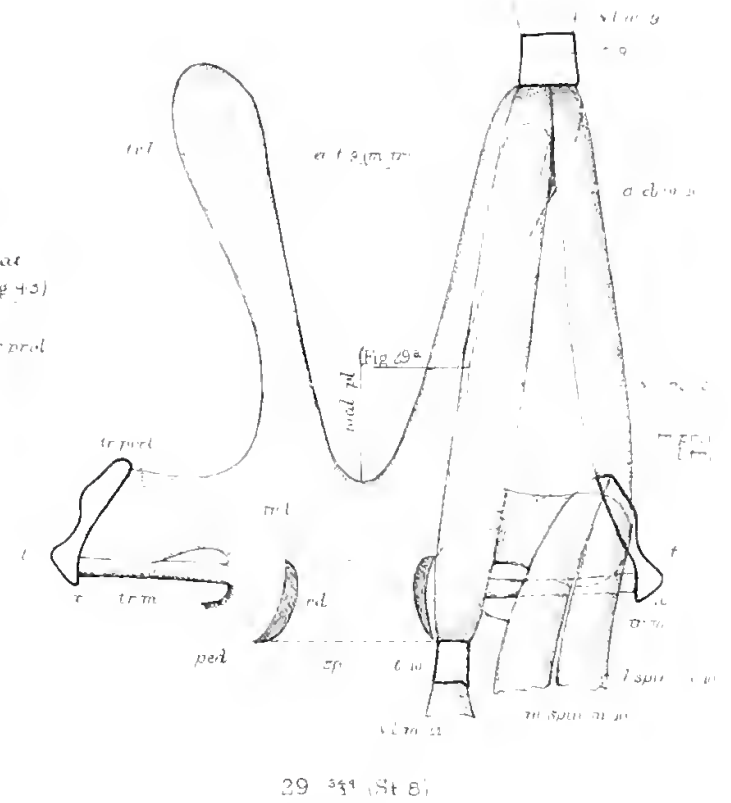
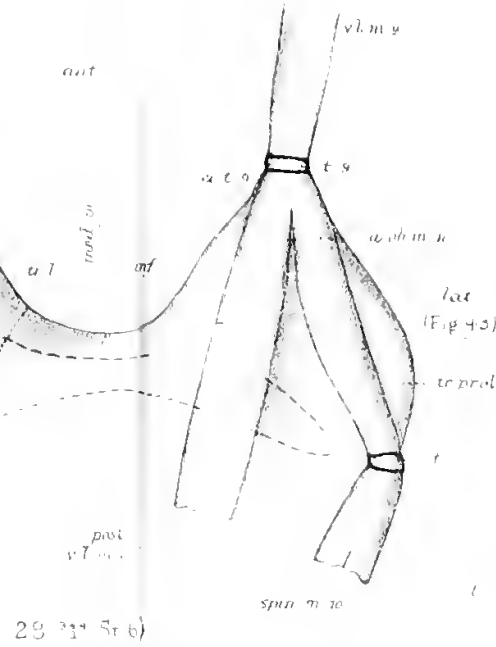
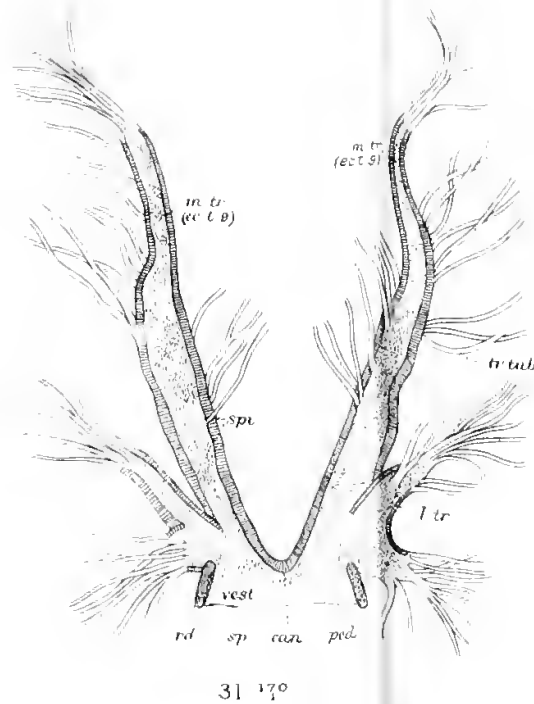
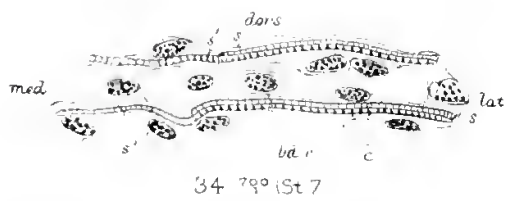
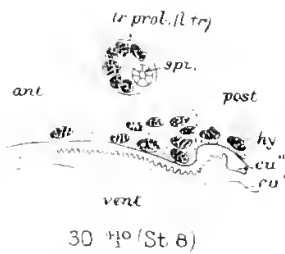
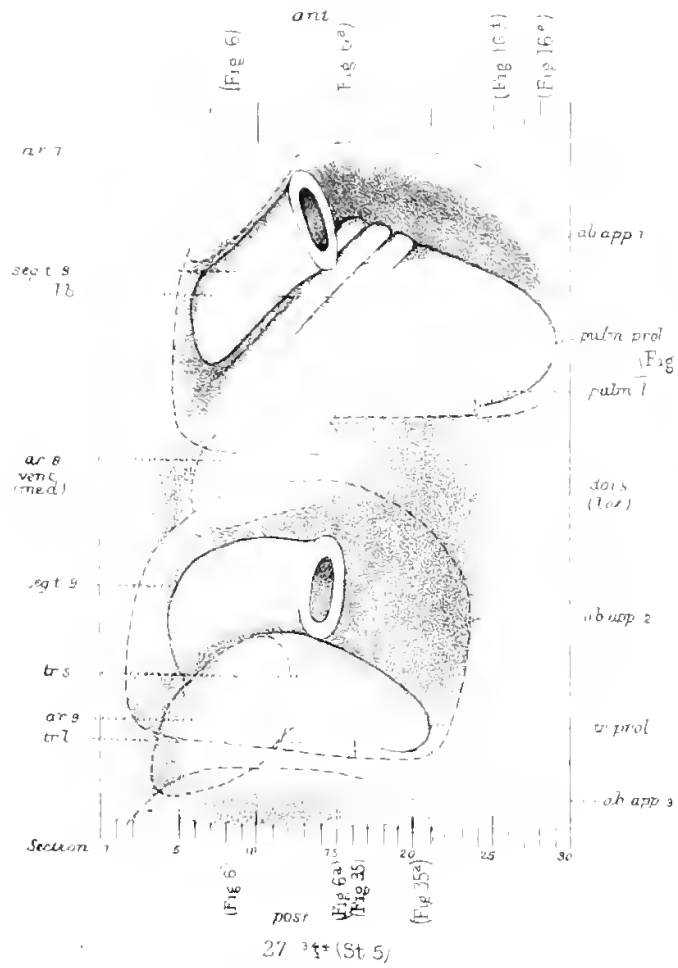
7. 1° (St. 1)



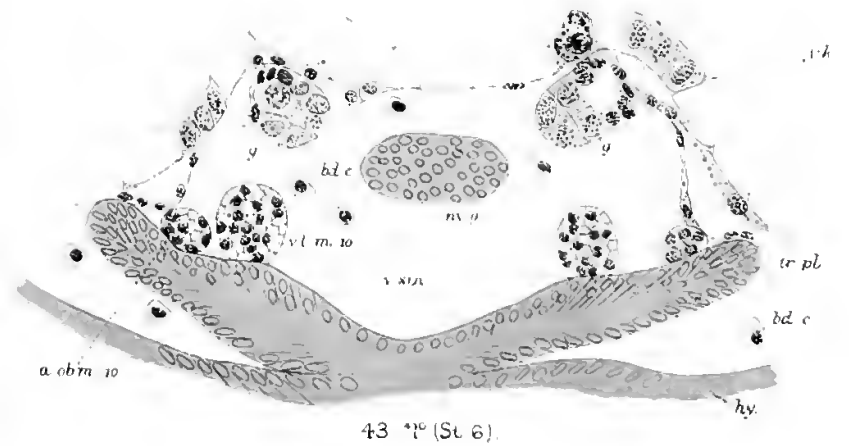
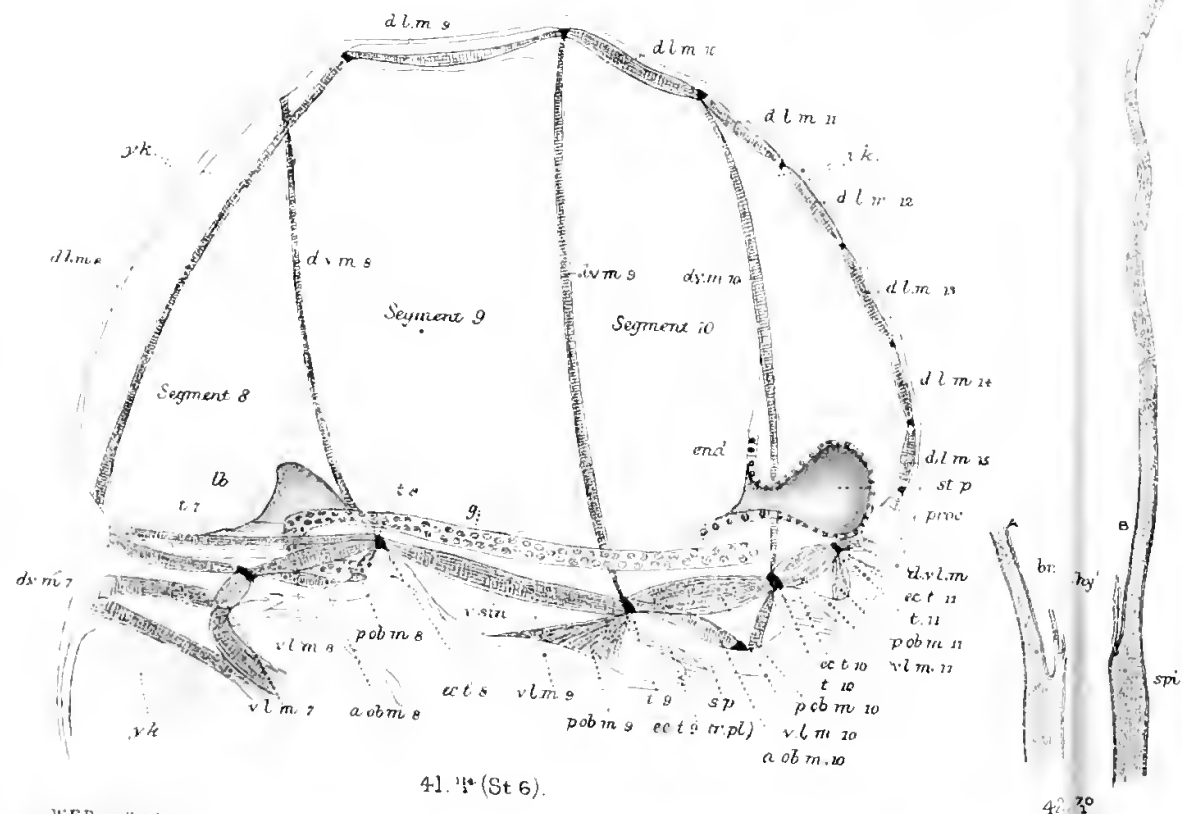
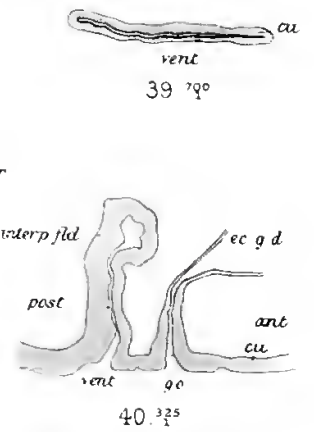
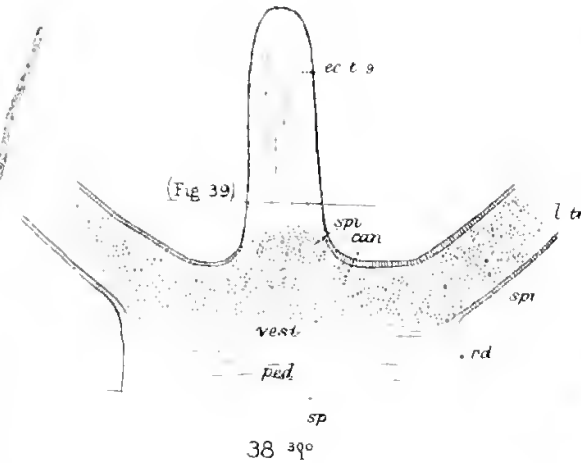
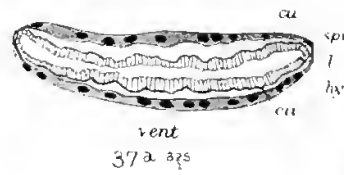
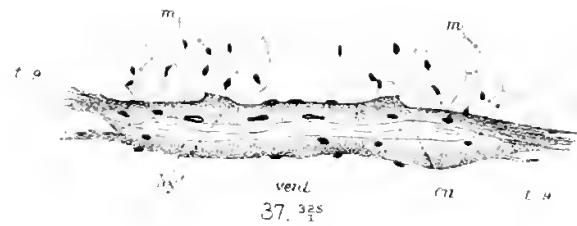
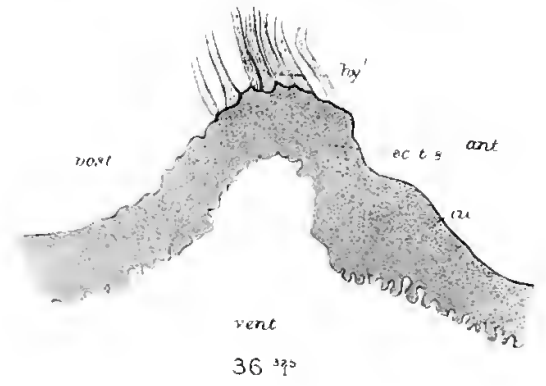
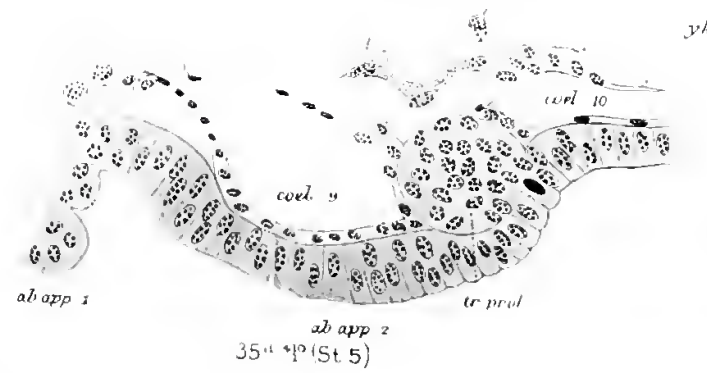
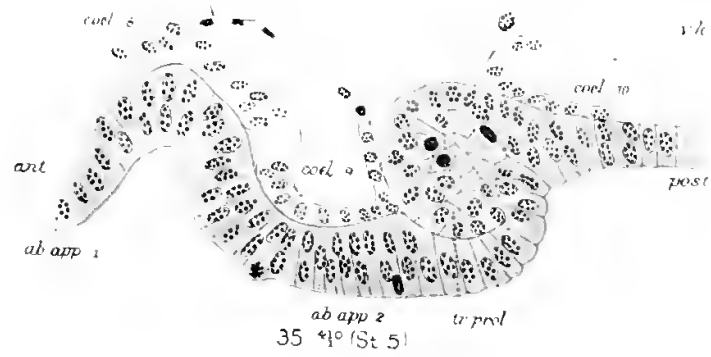
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Notes on the Nephridia of *Dinophilus* and of the Larvæ of *Polygordius*, *Echiurus*, and *Phoronis*.

By

E. S. Goodrich, F.R.S.,
Fellow of Merton College, Oxford.

With Plate 8.

IN this paper are recorded some observations made on four different types of true nephridia provided with closed internal ends bearing some form of solenocyte. Although all these nephridia have been more or less completely described at some time or other by various authors, I am able to add some details, not without interest, which help to complete our knowledge of these organs.

DINOPHILUS.

The nephridia of this free-swimming Annelid have been described by Schmidt, Korschelt (7), Meyer (9), Harmer (5), Schinkewitsch (11), and Shearer (14). It is stated by Korschelt that in *D. apatris*, and by Meyer that in *D. gyrociliatus*, the internal extremity is blind, and ends in a flame cell. In Harmer's excellent account of *D. tæniatus* the eud is said to lie in a cavity near the gut, and to bear a ciliated appendage or knob forming the base of attachment for the flame-like bunch of cilia which beat down the lumen of the canal. Harmer is not positive as to the absence of an opening.

In 1906 Shearer made the interesting discovery that the "ciliated appendage" of Harmer is really formed of a number of solenocyte tubes, comparable to those I have described in

many Polychaetes. These tubes had already been indistinctly seen and described by Harmer as "elongated, pear-shaped bodies" which "vibrated individually."

The blind internal end of the nephridium of *D. tæniatus* ends in a bundle of blind, slender tubular extensions, at the extremity of each of which is attached a long flagellum. The flagellum works down the tubule into the lumen of the nephridial canal, which is not otherwise ciliated.

While able fully to confirm these important observations made by Shearer on the living worm, I am also in a position to complete his description from sections of well-preserved material.

Fig. 1 is a slightly diagrammatic reconstruction, from sections, of the end of the nephridium. These structures are very small, and can only be made out with the highest powers. The nephridial canal is relatively thick-walled; the cells of which it is composed are loaded with excretory globules and pierced by an intra-cellular lumen. Nuclei can be seen at rare intervals along its course. One conspicuous large nucleus is always found at the extreme end, which projects into a free space, supported by strands of mesenchymatous tissue. Here the nephridial lumen expands into a chamber bounded on one side by a thin wall from which arise the "solenocyte" tubes. They may be considered as hollow outgrowths of the wall. In sections they appear rather shorter and stouter than in the living worm; this is probably due to contraction of the preserved material. Each tube bears a little lump of protoplasm at its free extremity, from which starts the flagellum. No nuclei are found on or near the tubes themselves. The whole apparatus thus consists of a single cell bearing some twenty or thirty tubes and flagella, all controlled by the one large nucleus mentioned above.

It is clear that in *Dinophilus* we have a new type of solenocyte formation representing perhaps an intermediate step between the Platyhelminth "flame-cell" and the more typical Polychaete solenocyte, in which each tube, with its

flagellum, has its own nucleus. This latter state might be reached by a multiplication of the nuclei until they came to correspond in number with the tubes. That *Dinophilus* is related to *Polygordius* has long been suspected; it is therefore interesting to note that in the larval nephridium of *Polygordius* there is a similar multiplicity of tubes, as I showed some years ago (2).

THE LARVA OF ECHIURUS.

It is to Hatschek that we owe the first detailed description of the larval nephridia of *Echiurus* (6). According to his account there are a pair of larval nephridia without internal opening. In the late larva there is a membrane separating the coelom near the gut from a space below the body-wall traversed by mesenchymatous strands. Hatschek describes the nephridium as passing inwards from its external pore through this space, to spread out in a system of fine branches on the outer surface of the membrane. The nephridium ends in "ein Büschel von Endorganen," and "die Endknöpfchen der feinsten Canälchen je einen Zellkern enthalten."

This description and the accompanying figures had long led me to suspect that the "Endknöpfchen" were in reality "solenocytes." It was not, however, till this spring that I had an opportunity of confirming my suspicions by the examination of living larvæ at Naples.

Fig. 3 is a careful drawing from life of the nephridium of a larva of about the age shown in Hatschek's fig. 4. A glance at my figure shows that the nephridium is, in fact, provided with typical solenocytes. The canal leading from the external pore has granular, rather thick walls; when it reaches the membrane outside the coelomic epithelium it divides into a number of thin-walled branches, which spread over the membrane. These terminate in delicate, almost cylindrical tubes, at the end of which are the nucleated cell-bodies. The nuclei can be seen even in the living state.

As figured by Hatschek, fine protoplasmic threads extend

from the cells, by means of which they are attached to the surrounding structures. From each of the cell-bodies a long flagellum passes down the tube into the canal of the nephridium almost to the external pore. A few similar but shorter flagella spring also from the wall of the canal itself. Sections show that the lumen is intra-cellular, that nuclei are present in the wall of the duct leading to the pore, but absent in the fine branches. It is interesting to find that the solenocytes project freely both into the mesenchymatous spaces on the outside of the membrane, and into the coelom, passing through the coelomic epithelium.

We may now add the Echiuroidea to the already long list of groups in which are found typical solenocytes.

What may be the origin of the anterior kidney tubes of the adult Echiurus is still quite unknown. Obviously they are not derived from the larval organs, which open much farther forwards in front of the large paired setæ. Hatschek (6) and Salensky (10) have given some account of the development of the posterior so-called anal kidneys, and these appear to be not nephridia at all, but coelomoducts derived from the coelomic epithelium. The more anterior kidneys of the adult are probably of the same nature. I may add that I have found no trace of the supposed opening of the duct of the larval nephridium into the coelom mentioned by Salensky.

THE ACTINOTROCHA LARVA OF PHORONIS.

In a paper published in 1903 (3) I was able to show that the larval nephridia of this larva project into the hæmocœle, where they end blindly in bunches of solenocytes. It is unnecessary for me to repeat here this account, or to again refer to the older literature on the subject. But attention may be drawn to several papers since published. Cowles (1) and De Selys Longchamps (12) have confirmed most of my statements with regard to the structure of the nephridium. Moreover, De Selys and Cowles agree with Ikeda (8) as to the origin of the nephridia from an ectodermal pit. But it

is to Shearer (13) that we owe the first clear and definite account of the development of the solenocytes themselves. They arise from the wall of the ectodermal pit, as is convincingly shown in his figures.

During a visit to Helgoland last year I was able again to study the nephridium in a living *Actinotrocha* larva (*A. branchiata*). In this form it is a large organ with a branched internal end lying in the preseptal hæmocœle. As shown in fig. 4, multitudes of solenocytes project from the wall of the nephridial canal. The nuclei are placed, not at the extreme end of each tube, but rather at the side. Now it is well known that an accumulation of blood-corpuscles is generally found in the immediate neighbourhood of the nephridium in these larvæ, and the interesting new point I have to bring forward is this—that very long, stiff cilia are set among the solenocytes, attached by their base to the wall of the nephridium between the solenocyte tubes.

Although I did not observe these cilia move very actively in the larva compressed under a cover-slip, yet I have little doubt that they agitate the blood-fluid, and so lead to the gathering of the corpuscles just mentioned. I have described almost exactly similar cilia on the nephridia of the *Alciopids* (2), but in these *Polychætes* they lie, of course, in the cœlom. *Actinotrocha* is the only form, so far as I know, in which they occur on a nephridium projecting into a hæmocœlic space.

THE LARVA OF POLYGORDIUS.

Since the figure of the nephridium of a larva from Ceylon published in 1900 (2) was based on somewhat doubtful observations made on scanty material, I have here given a new and more complete representation of the whole organ (fig. 2) as it occurs in the Neapolitan species. The drawing was carefully made from the living larvæ of about the stage shown in fig. 8, Pl. 11, of Fraipont's well-known monograph (4). It will be seen at once that the new figure confirms in every respect the account I previously gave, in which it was first shown

that the blind internal extremities are provided with solenocyte tubes.

The stiff refringent solenocyte-tubes run free from the protoplasm of the umbrella-like web, and are attached to it only at one end, which may be called the outer end. The opposite end of the tube plunges through the wall of the nephridial canal, into which it opens. The flagellum passing down the tube into the nephridial lumen is of remarkable length. Thickened protoplasmic ridges pass radially along the web from the central mass to the periphery, where they embrace the outer extremities of the tubes. The apparatus may be either expanded or contracted, as can be seen in the figure. In the expanded condition the web is flattened out with the tubes widely diverging; the central mass and contained single nucleus then lies somewhat flattened in the middle. When the web closes up, the tubes are on the contrary drawn together until they become almost parallel, and the central mass with its nucleus is made to bulge outwards as a convex knob.

There is no doubt, then, that in *P. neapolitanus* a single nucleus, at the tip of each branch of the nephridial canal, controls a set of from six to seven solenocyte tubes. If Woltereck's recent description of the nephridium of a North-sea larva is correct, it would appear to differ considerably in structure; for he states that there is a nucleus to each tube, that the tubes are covered by the cytoplasm, and figures no web between them (16). In an important paper on the development of *Polygordius*, Shearer (15) has given a most careful account of the origin of the larval nephridia; they arise from two cells differentiated quite early, lying on the inner surface of the ectoderm, and probably derived from it. Each of these cells multiplies to form a chain which develops into the whole nephridium. The solenocytes arise from the extremity of the nephridium itself. These observations entirely confirm the view that the canal and solenocytes of the Annelid nephridium form a whole, a single organ derived from one rudiment, strictly comparable to the canal and flame-cells of the platyhelminth excretory organ.

May 12th, 1909.

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EXPLANATION OF PLATE 8,

Illustrating Mr. E. S. Goodrich's "Notes on the Nephridia of *Dinophilus* and the Larvæ of *Polygordius*, *Echiurus*, and *Phoronis*."

REFERENCE LETTERS.

b. c. Blood-corpuscles. *c.* Cilium. *c. m.* Central mass of cytoplasm containing a single nucleus. *c. t.* Connective tissue. *fe.* Flagellum inside the solenocyte tube. *n.* Nucleus. *n. c.* Nephridial canal. *np.* Nephridiopore. *pr.* Cytoplasmic process. *prm.* Mass of cytoplasm at end of tube. *s.* Septum separating the cœlom from the hæmocœle in which lie the solenocytes. *t.* Tube of solenocyte. *w.* Thin cytoplasmic web.

FIG. 1.—Restoration from sections of the inner end of the nephridium of *Dinophilus tæniatus*. The organ is represented as if cut longitudinally.

FIG. 2.—Drawing from life of the whole nephridium of the larva of *Polygordius neapolitanus*.

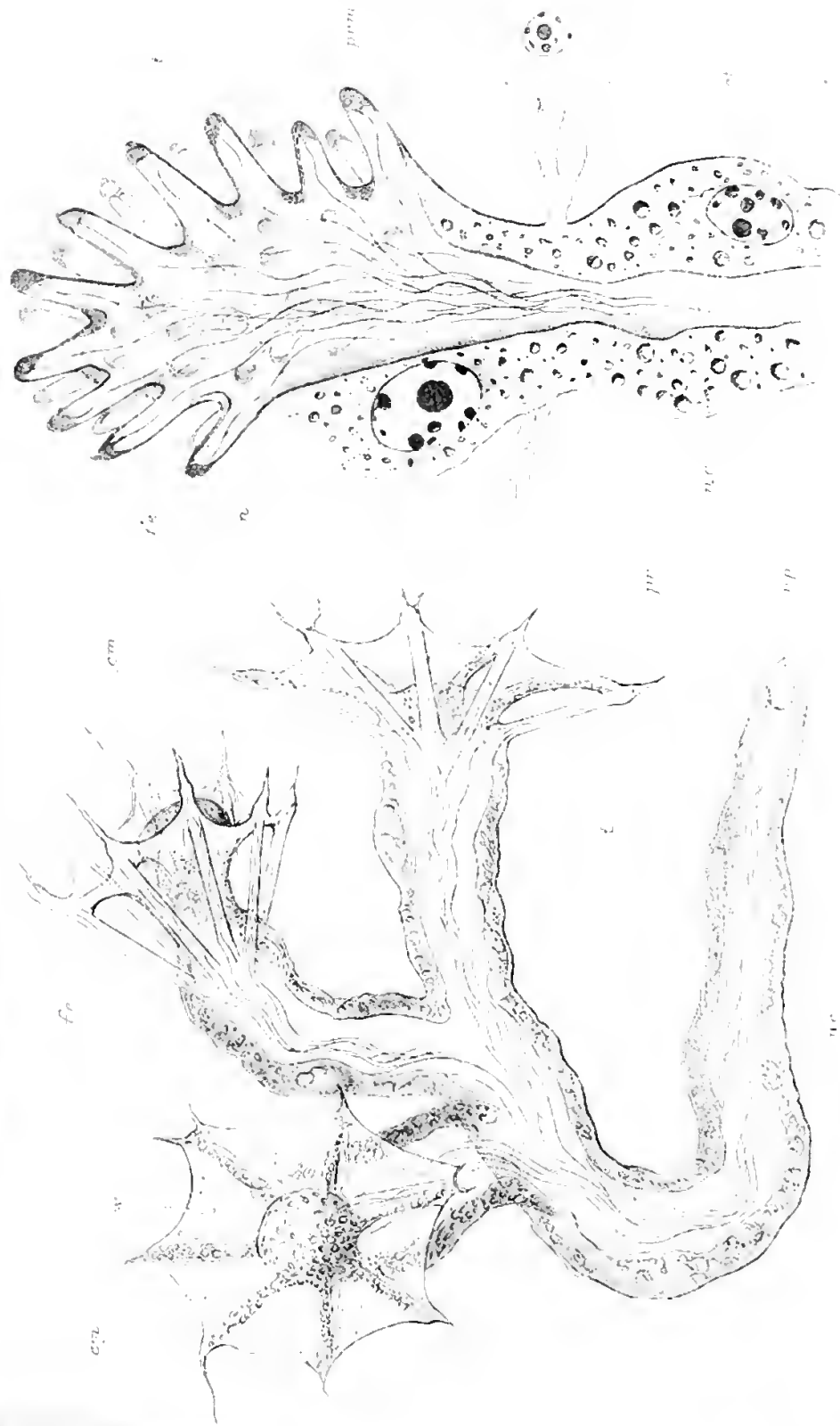
FIG. 3.—Drawing from life of the whole nephridium of *Echiurus* sp.

FIG. 4.—Drawing from life of the inner end of the nephridium of *Actinotrocha branchiata*.



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**Further Notes on a Trypanosome found in the
Alimentary Tract of *Pontobdella*
muricata.**

By

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Carnegie Research Fellow; Junior Assistant to the Professor of
Protozoology in the University of London.

With Plate 9 and 5 Text-figures.

INTRODUCTION.

IN a previous paper published in the 'Proceedings of the Royal Phys. Soc. of Edinburgh,' vol. xvii, No. 3, 1907, an account was given of the main features in the life cycle of *T. raia* as it appeared in the leech *Pontobdella muricata*.

I could not, however, at that time get clear knowledge of the early stages of the infection in the leech, nor could I get conclusive evidence that the form appearing in *Pontobdella* was really *Trypanosoma raia*. The following observations fill in these important gaps, and appear to me to make it clear that the flagellates found in such abundance in this leech are derived from the skate and form part of the life cycle of *T. raia*.

I have also taken the opportunity afforded me by a fresh set of infected leeches of again working through many of the stages in that host. On this occasion I have abandoned the Giemsa method and the dry fixation of the films, and have used instead such fixatives as Schaudinn's fluid, Hermann

and Flemming's fluid. The stains used were iron hæmatoxylin, Delafield's hæmatoxylin, Twort's combination of licht-grün and neutral red, and acid fuchsin. These are all useful stains. The Giemsa dry film method has been so much criticised that one felt that cytological work carried out with this stain was on an insecure basis until confirmed or criticised by other methods.

The corrections in this paper apply only to cytological detail, and my view of the life cycle, as a whole, has only received further amplification from the new facts at my disposal.

In the spring of 1907 I received a number of *Pontobdella* cocoons from the Marine Station at Plymouth; they had been deposited on clam shells. I put them into a glass jar filled with clean sea water, covered it with a loosely-fitting glass lid, and simply put it on to a shelf in the laboratory. I went to Ceylon in the course of the summer, and was later informed that the leeches hatched out about the beginning of October, 1907, although the exact date was not noted. On my return in the autumn of 1908 they were still alive in the original jar and the same water. In November I took them to the Marine Station at Millport,¹ on the Clyde, and fed them on infected skate to obtain the first stages of the parasite in the leech.

I may say in passing that the Trypanosome infection is very common in the skate caught in the Clyde area; more than 50 per cent. of these fishes are infected, a circumstance which accounts for the great frequency of the parasite in the leech, only occasional specimens being uninfected.

The leeches, it was observed, showed the greatest excitement—waving about actively in all directions—when a shadow was made to fall upon the jar containing them. This reaction to the appearance of a shadow is very marked indeed, and must be of service to the creature in its natural state. The

¹ I wish to express my thanks to the director of the station for affording me all possible facilities in carrying out these experiments.

skate, coming over the ground upon which a leech is attached, will, of course, cast a shadow, and this will be one of the ways the leech becomes aware of the presence of its host.

The young leeches attached themselves readily enough to the skate, except in one or two cases where they required a good deal of coaxing; ultimately, however, they all fed.

The *Pontobdella* apparently does not feed at once, possibly on account of the difficulty in piercing the skin. The attitude of the leech when attaching itself with the intention of feeding is very characteristic. It takes firm hold with its posterior sucker, and then bends its anterior sucker in so that it is touching its own body. It then slides the anterior sucker down along the body, finally attaching it to the skate at a spot close beside the posterior sucker. These leeches made quite a large wound for such small creatures.

In some cases, after feeding for a time, the leech detaches the anterior sucker, but does not, as a rule, remove the posterior one. Finally, after a greater or less interval of time has elapsed, the feeding is resumed. On one occasion I was able to observe the process very clearly. A leech was put on to a skate at 2.30 p.m., on the 14th November, 1908; at 10 a.m. on November 15th it was attached by the posterior sucker only. The leech had fed, but had not taken very much (the leech is, of course, rather transparent when young, and it is possible to tell at a glance if it has fed or not). Three quarters of an hour later it was still in the same position, with the anterior sucker free. I then held the skate under water in the tank in such an attitude that I could see the leech, which had begun to make searching movements with the anterior sucker. It presently slid the anterior sucker down its body and on to the skate, but moved it about until it passed over the old wound, when it buried its sucker in it. The leech seems to force its proboscis in pretty deep, judging from the wound and its attitude while sucking. At 7 p.m. on the 15th the leech was again only attached by its posterior sucker, but it was much swollen, and had blood right up to the anterior end. It was left attached to the

skate till the morning of the 16th, but did not seem to feed again.

Anyone who has reviewed a large number of a given species of leech will have observed that there is a good deal of individual variation in the processes of digestion. The broad lines are of course the same, but there is always a certain amount of individual idiosyncrasy. This in *Pontobdella* is chiefly expressed in the greater or less fluidity of the blood in the crop and the nature of the bacterial flora moulds, schizomycetes, etc., present. These complex circumstances no doubt react upon the Trypanosomes and may explain a certain variability in the detail and also in the time co-efficient of some of the developments.

The blood in the crop of *Pontobdella* has a tendency to coagulate. It forms a rather dry mass with fluid in the interstices. The time factor in this stiffening of the blood is rather variable. It always occurs, but the time at which it happens and the length to which it goes differs a good deal in individual specimens. Late in digestion the mass in the crop tends to become fluid again.

In *Pontobdella* the crop is a single rather thin-walled sac passing back from the œsophagus right to the posterior end of the body. The opening from the crop into the intestine is placed at a point about two thirds of the way from the anterior end and it passes back as a narrow tube lying on top of the crop.

The young *Pontobdella* which had been hatched in captivity were opened at different intervals after feeding on infected skate.

The condition of the parasites was carefully observed and the following course of development was found to take place. It must be observed in passing that skate's blood contains an immense number of leucocytes, which present very confusing appearances; also the parasites are rather scarce until the multiplication period sets in. Some searching is therefore required to find the Trypanosomes in the earliest stages.

A leech (5a) was put on to an infected skate at 9.30 p.m. one evening; 12 hours later it was feeding, and had already ingested a good deal of blood; $2\frac{1}{2}$ hours later it had ceased feeding. The leech was opened $17\frac{1}{2}$ hours after it had been first put on to the skate, and $3\frac{1}{2}$ after it had finished feeding. So that the earliest ingested Trypanosomes had been in the leech about 16—17 hours, and the last ingested ones about 3—4 hours.

The blood was very fresh-looking, and no obvious changes

TEXT-FIG. 1.



Drawing of live Trypanosome from the crop of *Pontobdella*.
The animal, which has been recently ingested with the blood,
is in process of rounding off.

had taken place in the blood corpuscles. The Trypanosomes showed very variable appearances. A good number still showed the flagellum, but were no longer in the typical Trypaniform condition. For the most part, they were somewhat pyriform (text-fig. 1), with an immensely long, thick flagellum protruding from one end. Some very fantastic appearances were seen where the body of the Trypanosome had assumed an irregular shape with curious rounded bulges, and where the flagellum had broken loose from the membrane, and had become tangled round the body, the end was usually free

and still motile. In other cases free flagella still actively motile were seen; this has often enough been observed with Trypanosomes, but in this case the flagellum does not take the kinetonucleus with it. Uninucleate stages of this parasite have never been seen. One of these free motile flagella was seen to become secondarily attached to a resting individual. This animal was watched for many hours in case the process might prove to be of more than merely casual significance, but no development took place. Besides these flagellate creatures, others were present which had already discarded the flagellum (text-fig. 4). These were rounded egg- or pear-shaped individuals with a characteristic clearly visible nucleus. It is composed of a softly refractile circular body surrounded by a bright halo. The nucleus lies towards the broader end of the body. These animals present a very characteristic appearance, but, nevertheless, are easily overlooked in the mass of leucocytes and blood-corpuscles.

These non-flagellate organisms were already in a few instances undergoing division but no sign of the new flagellum was as yet forthcoming.

Another leech (7*a*) opened forty-eight hours after it began to feed showed only resting forms. The blood in the crop had coagulated into a rather dry mass but the corpuscles showed no signs of degeneration.

These resting stages of the Trypanosome are identical in appearance with those so frequently seen in *Pontobdella* found infected in nature. A resting pear-shaped individual was chosen as a subject for observation at 4.30 p.m. When it was first observed¹ the trophonucleus was clearly visible and had its usual appearance of a sphere surrounded by a halo. Half an hour later the animal was more rounded, the nucleus was less distinct, and a slight groove had appeared at the broad end. By 5.30 the nucleus, as such, had disappeared, but a large clear oval space had appeared in its stead. At about 5.50 the two nuclei began to reappear, joined by a

¹ I am indebted to Mr. C. H. Martin for kind assistance in carrying out some of these continuous observations upon the live specimens.

clear area. At 6.15 the two nuclei were once more quite clearly defined but the clear area joining them remained visible till about 9 o'clock after which it was no longer to be detected. This clear band is the remains of the division spindle: it is a very characteristic feature in the stained specimens. During the division of the nucleus the body had gradually become flattened in an antero-posterior direction and correspondingly widened laterally. Grooves also began to appear in the antero-posterior direction. It is to be noted that these arose both at the anterior and the posterior end—the anterior end being the broad end at which the nucleus lies when the animal is in the pear-shaped condition and at which the flagellum is later developed.

These grooves altered a good deal in appearance during the next few hours and towards 4 a.m. had deepened till the animal presented the picture of two pears stuck together in the middle, with however, the two broad ends and the two pointed ends free. This is a point of some slight importance. When in the trypaniform state division of the protoplasm of this parasite usually begins from the anterior end and proceeds to the posterior. This is likewise the rule in the crithidial state. This question of the grooves arising at both the ends of the parasite is not in itself deserving of much remark but it explains some curious stages where division of the protoplasm goes from the posterior to the anterior end, to be described in a later part of the paper.

This individual was watched for another two hours and one of the two daughter-individuals developed a stiff and very short flagellum which, however, showed no signs of movement. The other individual appeared to have a little projection suggesting a flagellar rudiment. At 6.30 a.m. the animal was finally abandoned, although the complete separation of the protoplasm had not yet occurred.

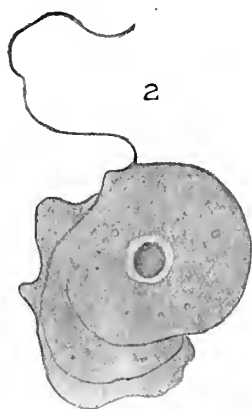
The flagellum seems to appear for the first time somewhere between the second and third day after feeding. It is a very characteristic feature that it generally arises at a division stage. The flagellum is at first a stiff and relatively thick

little rod which sticks straight out from the anterior end of the organism. A very considerable time seems to elapse before it becomes motile, I cannot say exactly how long, but it seems to be more than twelve hours.

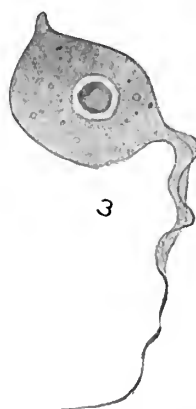
A leech (6a) opened six days after feeding showed a typical infection of the varied type so characteristic of *Pontobdella*.

True Trypanosomes had already appeared, some of these

TEXT-FIG. 2.



TEXT-FIG. 3.



Trypanosomes from the blood of the skate rounding off on a sealed slide. The drawings are from live specimens.

were broad individuals and others were much more slender, but not of the elongated type which appears at a much later stage of digestion. The broad and slender types were joined up by innumerable intermediate forms. Besides these crithidial forms were also present. I call crithidial forms those where an undulating membrane is developed, but which have not the typical Trypanosome arrangement of the kineto- and tropho-nucleus. Herpetomonad forms with the flagellum sticking straight out from the broad end of the body and with as yet no membrane were also to be seen. And finally

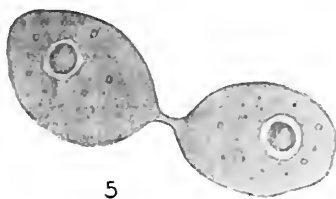
many rounded forms, some dividing and some developing the flagellum, were likewise present. Some of the trypaniform individuals were already dividing.

Conjugation was very carefully searched for as it seemed probable that it might occur at this stage of the life cycle, but no signs of such a process were detected. Two individuals were found joined by their posterior ends, one slightly broader than the other. They were watched continuously from 6 p.m. till 3.15 a.m., and the protoplasmic junction between the two was seen to become much more slender and pulled out, showing that the individuals were dividing.

TEXT-FIG. 4.



TEXT-FIG. 5.



TEXT-FIG. 4.—Rounded off Trypanosome.

TEXT-FIG. 5.—Division of resting Trypanosome.

Drawings made from live specimens in a sealed slide of skate's blood.

An interesting corroboration of the stages above described was obtained from blood drawn from a skate and sealed up between a coverslip and slide (text-figs. 2—5). A Trypanosome was continuously watched from 2.45 p.m. when the slide was made. At 4.30 the animal had come to rest. The flagellum which, when it breaks free from the membrane, is seen to be of a relatively immense length was tangled up round the animal. The slide was watched for some hours longer, but as the Trypanosome was no longer motile it was left. Next morning it was found to have divided into two.

The behaviour of the Trypanosomes on a sealed slide is interesting—a number do not alter at all, others very soon after the slide is made begin to react to the altered condi-

tions. They adopt a dumpy spiral shape, or the posterior end may become much thickened at the expense of the rest of the body (text-figs. 2 and 3). Sometimes the most fantastic shapes are seen, finally the flagellum breaks free but may remain attached to the Trypanosome by its posterior end. It may then become tangled round the body and stick out in stiff loops. The Trypanosomes in these phases on the sealed slide made from the skate's blood are absolutely identical in appearance with the motile forms described in leech 5a (cf. text-fig. 1). The time at which the Trypanosomes come to rest varies much.

On another occasion a rounded non-motile Trypanosome which had discarded its flagellum was chosen for continuous observation on a slide of skate's blood which had been mounted for fourteen hours. The animal was seen to divide into two about 11 o'clock in the forenoon. During the afternoon, at about 4 o'clock, these two individuals each divided thus forming four little rounded animals lying more or less in contact. By 7 the same evening they had become more oval and were identical in appearance with the resting phases in the leech. By 9.30 p.m. short projections were seen at the broad end of two out of the four creatures under observation. The slide was left about 10 o'clock that night as the animals were not motile. Next morning at 9.30—thirty-six hours after mounting the slide, observation was again resumed and it was found that the creatures had each divided. The eight resulting individuals were still closely apposed but not connected with each other. Unfortunately these creatures were lost owing to a careless movement. The slide was, however, in the following condition. Unaltered Trypanosomes, still actively moving, were to be seen, non-motile groups of four and also a few groups of six and eight individuals were present.

I had often noticed that the Trypanosomes on a sealed slide of skate's blood altered their shape, but, thinking this was merely a pathological manifestation, had not persevered with the observation. The process is easily enough passed over

unless the observation is continuous, as the infections are generally rather slight, and, once the Trypanosome has come to rest, it is not quite a simple matter to see it among the large number of leucocytes and red corpuscles. Moreover, the curious fact that all the Trypanosomes on a slide do not round off leads one to imagine that no development has taken place.

A low temperature seems to favour the process. This work on the live skate's blood was carried out at the Millport Marine Station, and the most successful set of observations was obtained in very cold weather, when the temperature of the laboratory was much lower than usual.

It appears to me that these experiments with the young leeches, which confirm Brumpt's short sketch of the life cycle of *T. raia*, are good evidence that the Trypanosome in *Pontobdella* is *T. raia*. Of course it may be urged by those who do not consider that Trypanosomes have a cycle of evolution, however simple, outside the vertebrate host, that the young leeches were hereditarily infected with a flagellate of their own. Against this I can only advance that the long fast of more than a year, to which these newly hatched leeches were subjected, is not in favour of the survival of such parasites; and that the close correspondence between the early stages in the leech, and those obtained upon the sealed slide from the skate, is strongly in favour of the identity of the parasites. Further, even if, in addition to the direct infection from the skate, a hereditary infection also exists in *Pontobdella*, it has still to be proved that the form inherited is not *T. raia*.

The only way in which quite conclusive proof of the question could be obtained is by means of experiments with both leeches and skate hatched in captivity. I hope ultimately to be able to carry these out, but the difficulties in the way are obvious, as the obtaining of *Pontobdella* cocoons is a matter of chance, and the breeding of skate takes time and is often uncertain.

I cannot agree with Capt. Patton and Mr. C. Strickland, who, on the basis of my previous description, consider this parasite to be an independent *Crithidia* having no connection with *T. raia*. In a recent paper¹ I observe that they place it in this group with the name of *C. robertsoni*. I would like to point out that, quite apart from the question as to whether this parasite belongs to the cycle of *T. raia* or not, it certainly is a Trypanosome. The *Crithidial*, like the *Herpetomonad* stage which precedes it, is transitory; the animal ultimately adopting the Trypanosome state. I admit at once that the distinction between a *Crithidia* and a Trypanosome is not a very important one, but, such as it is, I do not see that there is any scientific point in neglecting it.

I now wish to give a brief account of the parasite as seen in stained films, fixed, for the most part, in Schaudinn's (alcohol-acetic corrosive sublimate) fluid. The stains used were Delafield's hæmatoxylin, Heidenhain's iron hæmatoxylin, Twort's licht-grün, and neutral red combination and fuchsin.

Delafield gives an excellent result, staining the nuclear structures with clearness and precision; the flagellar apparatus stains, but does so a little faintly.

Iron hæmatoxylin gives a very clear picture, staining the nuclear parts coal black, and bringing into good relief flagellar and cytoplasmic detail. Great care, however, must be taken in the interpretation of this stain, as it leads one into much the same errors as Giemsa's method, in so far as it stains chromatic and acromatic structures alike. Therefore, while Heidenhain's method gives a really splendid picture, it is necessary to check the results by Delafield's hæmatoxylin, which is a much safer stain.

Twort's combination of neutral red and licht-grün was also used. This is a clear, transparent stain, giving a red reaction

¹ "A Critical Review of the Relation of Blood-sucking Invertebrates to the Life Cycles of the Trypanosomes of Vertebrates, etc.," by Captain Patton and C. Strickland. 'Parasitology,' vol. i, No. 4, Dec., 1908.

for chromatin, and a green reaction for cytoplasmic and achromatic structures. The drawbacks to this stain are the uncertainty of action which seems to attach to all delicate double stains, and the fact that there is another loophole for uncertainty in the process of washing out the stain; so that it remains doubtful in some cases whether the nuclear colour is absent from a structure, owing to the absence of chromatin or through the stain having been washed out.

Fuchsin gave quite good nuclear pictures, but did not bring up the flagellar apparatus sufficiently well.

The drawings in the plate are made from two well infected leeches found infected in nature. They were at the early part of what I have called the middle period of digestion.

For convenience sake the periods of infection may be divided into three, corresponding to the stage of digestion:

(1) The early stage, when the blood is just coagulating, and when the first signs of the dark green-brown fluid is visible in the upper part of the intestine. The parasite at this time is in the condition of throwing off the original flagellum and adopting the resting state, during which division begins to take place. The parasites are for the most part still in the crop.

(2) Middle period of digestion, when the intestine is full of the green-brown fluid, where breaking down of the blood is going on actively. The parasite is now in the intestine in large numbers. It shows the whole range of forms, from the spherical non-motile creature to the typical Trypanosome. Great variation in size and thickness of the parasites is to be observed. Very slender, long forms are only occasionally to be seen. This middle period is of very long duration.

(3) Final period of digestion, when the crop is empty (or almost so) of blood, and the intestine nearly, or completely, free from the characteristic green-brown fluid. The Trypanosomes are now long, slender forms, with the kinetocore in the typical Trypanosome position. The forms now begin to remount the crop, and are also to be found in a still more slender condition in the proboscis.

The drawings, being made from leeches in the earlier phase of period (2), do not show the long, slender Trypanosomes developed during period (3). This final stage of the Trypanosome is most striking; but it is, of course, a matter of chance to get a leech in this condition in nature. This year I only got one; it was only slightly infected, and I was unfortunate in not getting fixed films.

It will be convenient first to give an account of the Trypanosome phase as found in the intestine of the leech (figs. 8—11), and then to give any points of interest in its development from the resting form.

The protoplasm is finely and evenly granular without vacuoles; indications of alveolar structure can be detected in some specimens, but are not very conspicuous. Protoplasmic inclusions are only occasionally present.

The trophonucleus is composed of a large central karyosome surrounded by a wide halo, which is in turn surrounded by a "membrane." Fine, but perfectly distinct, rays pass from the karyosome to the outer membrane. The karyosome is quite obviously made up of two substances, namely, the chromatin and an achromatic substance, in which the chromatin lies embedded. This achromatic substance frequently receives the name of plastin, and, while this does not convey any very clear idea, it is nevertheless a convenient and useful term. In Delafield's preparations the plastin stains a pale greyish-blue, in iron hæmatoxylin it is brownish, and it takes the green colour in the Twort's combination. The nature of the rays is a little obscure: they stain, as a rule, rather faintly with Delafield, but in some cases take the colour more deeply; Heidenhain shows them up black, but they wash out easily. I am inclined to regard them as more of the nature of plastin, but they seem at times to carry chromatin. The membrane shows very often little condensations of chromatin-staining material at the points where the rays meet it. The membrane stains well with Delafield and also with fuchsin, likewise with Heidenhain, but washes out long before the karyosome. The condensations on the membrane appear to

me to be chromatin, but in Twort's stain they do not take up the red colour. I do not lay very much stress on this point, as it is just in a question of this kind that I think such a stain as Twort's is rather unreliable. There seems to be in the membrane, as in other parts of the nucleus, an underlying substance of an achromatic nature, in or on which the chromatin is deposited.

This nucleus is exceedingly constant in all the stages of the parasite as found in the leech, the only variation lying in slight differences in the condensation of the chromatin in the karyosome and the membrane.

The pictures presented in the dried Giemsa preparations differ greatly from this account. The most curious feature about this is that some of the Giemsa appearances give a very tolerably accurate representation, while others depart completely from the type shown by the wet method. The eight chromosomes so often seen in the Giemsa nuclei are not to be detected in the hæmatoxylin films. The rays and the condensations on the membrane are, I have no doubt, the manner in which these appear in the wet films. The number of the rays can, however, not be made out, as they are excessively fine, nor do the condensations on the membrane stand out sufficiently separately to be considered as individual structures. The Giemsa stain, of course, always increases the apparent size of any nuclear element into which it penetrates.

The kinetonucleus takes all the stains mentioned with great intensity; it is relatively large and rod-shaped. In close proximity, and apparently attached to it, lies the blepharoplast (Minchin, 'Quart. Journ. Micr. Sci.,' May, 1908, vol. 52), (figs. 9-10, etc.).

This structure will be more fully considered when the development of the flagellum is discussed. The blepharoplast stains with iron hæmatoxylin, but the stain is washed out more readily than from the nuclear structures; it appears grey-blue and rather faint with Delafield, and is difficult to detect at all in Twort preparations. The flagellum

runs forward from the blepharoplast, ending, as usual, in a free whip. It stains green with Twort's combination and faintly with Delafield—more deeply with iron hæmatoxylin. The undulating membrane is developed to a varying degree, but is never much frilled.

Two other structures remain to be described. The first is a small granule only staining in Heidenhain preparations. It lies posterior to the kinetonucleus and often near the posterior end of the body. Sometimes it appears to be connected to it by a delicate strand. This granule is also found in the Giemsa preparations and is clearest in the Trypanosome phase. It has possibly something to do with the anchoring of the kinetonucleus (figs. 9 and 10).

The second is an element which I have never seen in the dried preparations at all, but which is a pretty constant feature in the wet films and appears equally so with all the four stains used. Just anterior to the trophonucleus a small condensation is to be observed in the protoplasm surrounded by quite a definite little halo. In rather dark Heidenhain preparations it stains almost black (figs. 9 and 10), with Delafield it looks grey-blue with soft outlines, and is usually only very slightly darker than the surrounding protoplasm (figs. 8, 11 and 19). It stands out clearly in these films more by reason of the halo than on account of its greater depth of colour. In Fuchsin films it is very clear: it is also visible in Twort's preparations but does not take on the red colour. It sometimes appears to be double. The nature and function of this structure is quite obscure: it is most clearly visible in the Trypanosome stage and its position is quite constant, but it is also present in the earlier phases. The position of the kinetonucleus often obscures it in these and makes it difficult to see.

The Trypanosome just described arises, as has been said, from a rounded resting form which develops a flagellum (figs. 1—11). The body gradually elongates and the kinetonucleus migrates backwards until it is well posterior to the trophonucleus. An undulating membrane develops during this pro-

cess and the creature takes on the typical Trypanosome facies. The only point about this that calls for special attention is the development of the flagellum.

It may be noted in passing that, owing to the flattening of the Trypanosomes prepared by the dry method, certain details may be more distinctly visible in such specimens than in those prepared by the wet method.

The earliest stage of the development of the flagellum, of which one can be quite certain, is shown in fig. 2. Here it will be seen that two little projections have grown out from the kintonucleus which is itself in process of division. These little structures sometimes take the Heidenhain rather deeply; they are not, however, very easy to make out as any obliquity in the position of the kintonucleus is apt to obscure them. Later stages are shown in figs. 3—5. Here the flagellum appears as a thick strand arising from a granule with not very definite contours, which is in turn attached to the kintonucleus. This granule at the origin of the flagellum is the blepharoplast. The minute detail is not very clear in the wet preparations, but as far as I can make out the blepharoplast seems to be attached to the kintonucleus by a double thread. This may be seen in much later stages (figs. 5 and 6). The blepharoplast seems like the kintonucleus to be ultimately a thin rod-shaped body. It seems to arise from the kintonucleus, but I do not think in the light of its behaviour with the various stains that it is a chromatic body. It is true it often stains a deep sharp black with iron-hæmatoxylin, but that is no test for chromatin. It takes on a grey-blue pale colour with Delafield and shows up only dimly when at all with Fuchsin. In Twort's combination it stains green like the flagellum and is not very clear or precise. I am therefore inclined to regard the blepharoplast and flagellar apparatus which grows out from it as achromatic. Our knowledge of achromatic nuclear elements is so limited at present that it is impossible to say whether they can be regarded as an expression of the achromatic elements of the kintonucleus or not.

Giemsa's stain presents a greatly exaggerated picture of

the above development. The drying pulls the blepharoplast away from the kintonucleus and makes the thread joining them stand out very clearly; it also greatly enlarges the apparent size of the blepharoplast, and very markedly increases the flagellar rudiments, which are always thick at this stage. In addition to all this these flagellar structures stain a deep red corresponding to the chromatic colour. That such a stain should be a fruitful source of error is obvious. It must, however, in justice be said of this stain that it has done brilliant if unequal service to the advancement of our knowledge of blood parasites, and has, therefore, amply served its purpose.

The longitudinal division of the kintonucleus which I held to be the first stage in the development of the flagellum has been seen again in resting forms (fig. 1), but in the light of the above observations I do not feel confident as to how this appearance should be interpreted. It is in these, as in the other specimens of *Pontobdella* previously examined, a rare appearance, and the evidence for considering this as the initial step in the formation of the flagellum is very much less clear in the wet films than in Giemsa preparations. Moreover, I have recently found in *T. vittatæ* that the longitudinal division of the kintonucleus is the ordinary method instead of the transverse, as in *T. raiaæ*; it is, therefore, possible that the point may be open to some variation.

Division.—The figs. 7, 12—17 give pictures of various division stages, and it is perhaps in this point that Giemsa's method has, generally speaking, been the least misleading. The division starts as a rule by the transverse division of the elongated kintonucleus, but this point is open to slight variation.

The trophonucleus shows first an arranging of the chromatin in two masses within the karyosome (figs. 7 and 12). A well-developed spindle subsequently arises, but the chromatin is divided without the formation of an equatorial plate. The wet method brings up the spindle very well (figs. 13—17). Centrosomal functions of some kind seem exercised by the

condensations at the extreme points of the spindle, and the chromatin seems to pass along the "fibres"¹ from the two main central masses in such a stage, as fig. 13, to either poles of the spindle. There is at a slightly later stage (figs. 14 and 15), often a curious double appearance in the nuclei. Figs. 16 and 17 give the final stages. The spindle persists for some time after the nuclei are reformed, and can be clearly seen as a bright line in the live specimens.

Division occurs at all the different stages of development. There is an occasional not very well defined tendency to multiple division. Equal division is the rule, but unequal division is sometimes met with. Considerable variation in the division of the protoplasm is seen, as shown in figs. 18—24. It will be observed that in figs. 20—22 the division of the protoplasm has started at the posterior end instead of the anterior, as is more usual. Specimens of this type were watched for many hours in the live state in the hope that they might be individuals conjugating, but no evidence in favour of this was forthcoming, and I was led to believe that they are in all probability merely cases of division.

Some very curious appearances were observed where the protoplasm had split into several rod-like processes. This is shown in fig. 24, and, while not a common appearance, is still far too frequently seen to be dismissed as a casual abnormality. The figures, I may say, hardly do justice to all the varying sizes and shapes to be seen at this stage in the development.

As I have already said in a recent paper² on *T. vittata* from the soft tortoise *Emyda vittata*, we seem to have in these forms a type of life-history which is probably very widespread. The rounding off process and the subsequent development of a flagellate stage occurs, as has been recently shown in many of the Trypanosomes from cold-blooded hosts.

¹ I use the word fibre quite without prejudice. The word conveniently expresses the optical effect, and I have no means of knowing what they actually represent.

² 'Quart. Journ. Mier. Sci.' vol. 53, part 4.

It seems to occur in the Trypanosome from *Hyla arborea* (França, 'Bull. Soc. Portug. Sc. Nat.,' 1907), also in *T. granulorum* (França), also in *T. loricatum* (Dutton, Todd, and Toby, 'Ann. of Trop. Med. and Parasit.,' No. 3, 1907).

The similarity between the life-history of *T. vittatae* and *T. raiae* hardly needs emphasising. *T. raiae*, of course, shows clearly the adaptation to the peculiar habits of the *Pontobdella*, its relatively slow development and the long persistence of the resting phase being correlated with the stiffening of the blood in the crop and the very long period which the Trypanosome must pass in the leech owing to the extreme slowness of the digestion.

The work here recorded was carried out partly at the Millport Marine Station, partly in the Zoological Laboratories at Glasgow University and the Lister Institute, Chelsea.

LISTER INSTITUTE;
April, 1909.

EXPLANATION OF PLATE 9,

Illustrating Miss Muriel Robertson's paper on "Further Notes on a Trypanosome found in the Alimentary Tract of *Pontobdella muricata*."

Figs. 1—24 are drawn with 2 mm. apochr. immersion lens by Ziess, 1.40 N.A. long tube and oc. No. 12, with the assistance of the camera lucida. The magnification is approximately 4500 diameters.

Fig. 25 is drawn with the No. 2 eyepiece; the magnification is approximately 1600 diameters.

Figs. 1, 2, 3, 4, 8, 9, 10, 13, 15, 17, 18, 19, 21 and 23 are from Heidenhain's hamatox. preparations. The remaining figures are from Delafield preparations.

All the figures, with the exception of 25, are from the leech *Pontobdella*.

FIG. 1.—Resting phase, showing longitudinal division of the kinetonucleus.

FIG. 2.—Early stage in development of flagellum.

FIGS. 3, 4 AND 5.—Stages showing newly-formed flagellum.

FIGS. 6 AND 7.—Crithidial stages.

FIG. 8.—Trypanosome phase.

FIG. 9.—Trypanosome showing blepharoplast, granule at posterior end, and the structure just anterior to the trophonucleus.

FIGS. 10 AND 11.—Trypanosome phase.

FIG. 12.—Early division phase. Note condition of trophonucleus, kinetonucleus and blepharoplast.

FIG. 13.—Division stage showing spindle.

FIG. 14.—Later division stage.

FIG. 15.—Division stage showing trophonucleus spindle and also second division of the kinetonucleus. This is a rather unusual appearance.

FIGS. 16 AND 17.—Later division stages.

FIG. 18.—Division of Trypanosome.

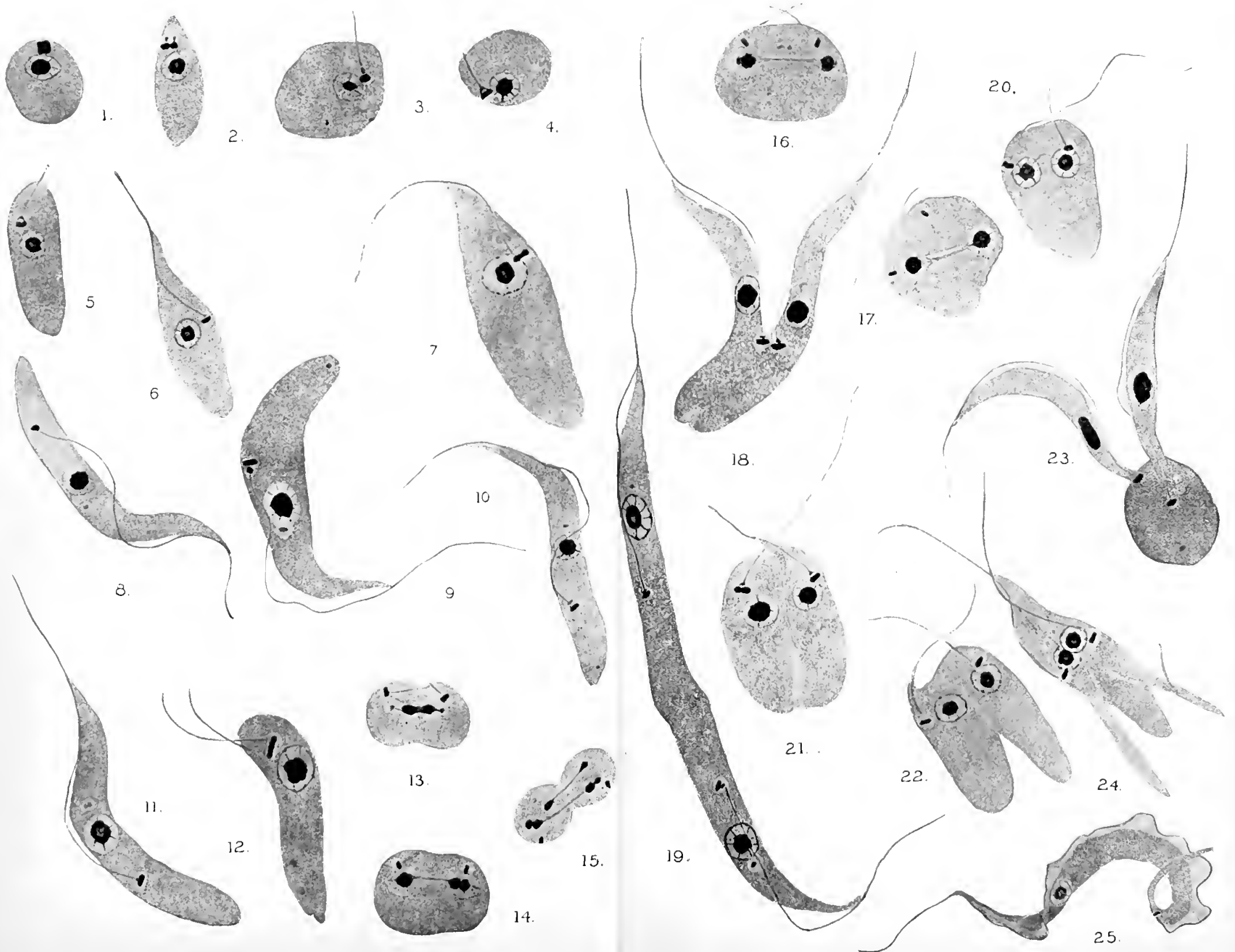
FIG. 19.—Division of Trypanosome.

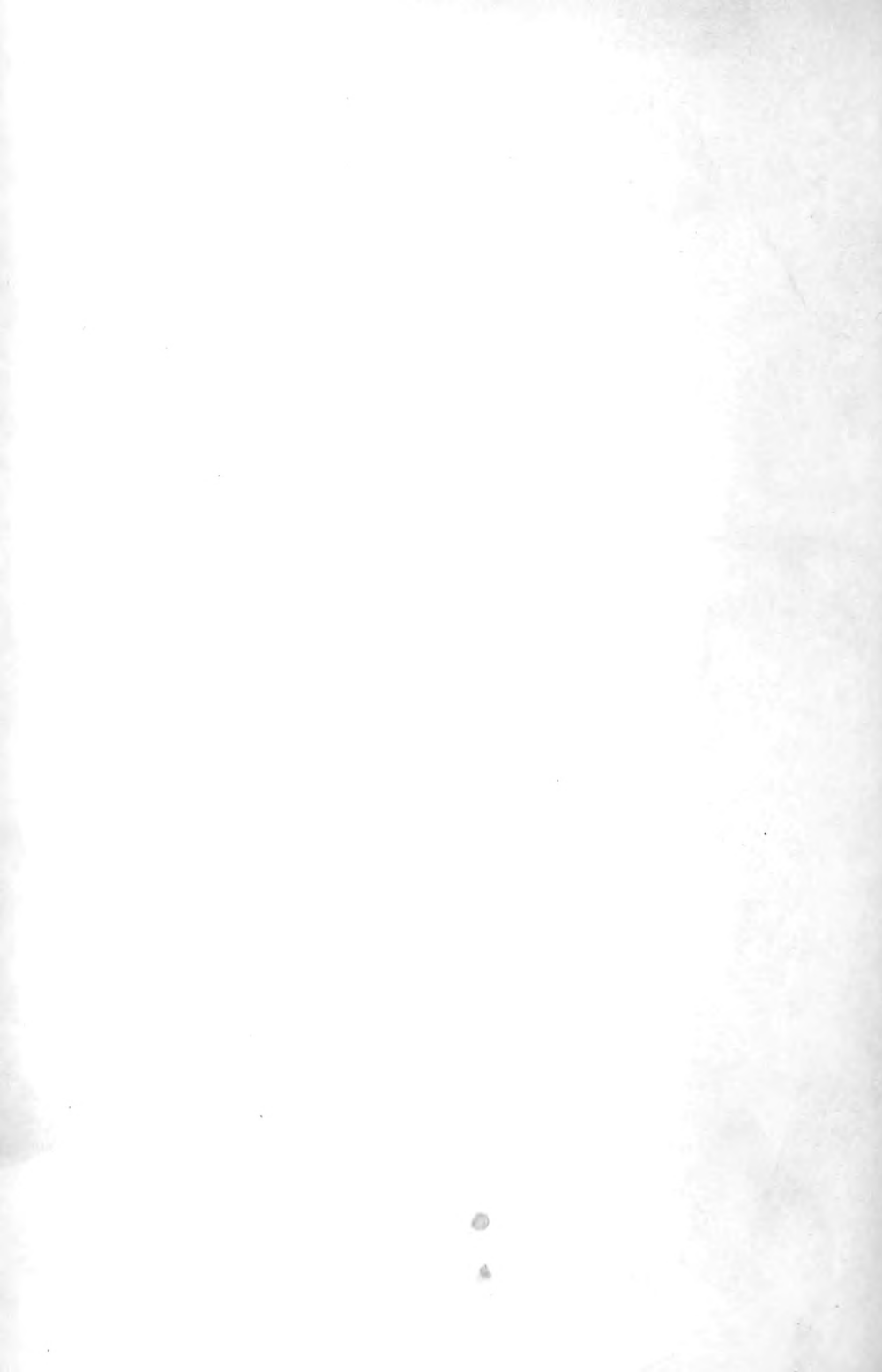
FIGS. 20-22.—Division stages where the protoplasm divides from the posterior end.

FIG. 23.—Rather unusual appearance where the posterior part of a dividing Trypanosome has formed a large rounded mass.

FIG. 24.—Division stage showing irregular splitting of the protoplasm.

FIG. 25.—*T. raia* from the skate's blood. Note this is drawn at a much smaller magnification ($\times 1600$) than remaining figures.





Dendrosoma Radians, Ehrenberg.

By

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and

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With Plate 10.

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Dendrosoma radians was first described by Ehrenberg in 1837 (9), but the figures he drew to illustrate its structure were not published until 1862 (11). In 1840 (10) he gave the following diagnosis of the species "Dendrosoma radians: D. corpusculis conicis, crassis, mollibus lævibusque, alterne ramosis, rame apice incrassatis et tentaculatis $\frac{1}{8}$."

Ehrenberg apparently saw the meganucleus, but in his first paper expressed the opinion that it was the male genital gland. In the figure (11, Plate III A) which he published later he represents a long axial vacuole, probably the meganucleus, and in the description of it apparently abandons the view that this structure is a genital gland.

Claparède and Lachmann (5) gave a good figure of *Dendrosoma* in 1861, but made a grave error in describing the meganucleus as an elongated contractile vacuole. There is no reason whatever for believing that there is in *Dendrosoma* any system of elongated canals in communication with the ordinary spherical contractile vacuoles.

The next important contribution to our knowledge of *Dendrosoma* is that of Levick (23), who in 1880 described and gave a good figure of the gemmule. Levick also gave a description of the interesting streaming movements of the protoplasm, which we have confirmed. There can be little doubt, however, that the "germ and sperm elements" of this author were not correctly interpreted. Notwithstanding the statement that he actually saw living spermatozoa comparable to those of *Hydra* discharged from the *Dendrosoma*, it is, in the light of our knowledge of the reproductive processes of other *Acinetaria*, impossible to accept the view that *Dendrosoma* possesses at any time definite male and female sexual glands.

In 1881 Saville Kent (19) published a more elaborate account of the species and gave a figure of a large specimen one tenth of an inch in height, which has been copied with some modifications in nearly all the text-books of zoology.

The principal new point of interest in Kent's account of the species is the description of "certain exogenously produced buds similar to those of *Acineta mystacina* described by Stein." If Kent's interpretation of these bodies were correct *Dendrosoma* would present the very remarkable peculiarity of producing two different kinds of gemmulae at the same time. Subsequent authors have referred to these bodies with caution: Bütschli (2) calls them "angebliche

freie äussere Knospen" ; Lankester (21), in his description of the figure, refers to them as "more minute reproductive (?) bodies."

There can be no doubt, however, that these bodies are not "buds" at all, but epizoic Acinetaria belonging to the genus *Urnula*.

The meganucleus was described by Kent as follows: "Endoplast ribbon-like, ramifying and much contorted in the stolon and basal portions of the main stem, continued as a simple band into the distal region and secondary branchlets."

He gives a figure of the meganucleus in a small portion of the basal part of an arm, which is probably correct so far as it goes, but in the figure of a distal portion of an arm the nucleus is shown to extend much nearer to the extremity than we have found it in any of our preparations. In the figure of the whole specimen the meganucleus is not shown at all. This figure has been copied by the writers of many text-books, and in some, as, for example, those of Calkins (3) and Lang (20), the meganucleus has been added to the figure and erroneously shown to extend to the extremity of all the branches.

The other genera of Acinetaria that are apparently closely related to *Dendrosoma* are *Trichophrya* (C. and L.), *Lernæophrya* (Perez), *Astrophrya* (Awerinzew), and *Dendrosomides* (Collin). Concerning *Astrophrya* we have at present only a short note and figure by Awerinzew (1). The single specimen obtained was found free in the plankton of the Volga, and, like some of our specimens of *Dendrosoma*, it had a massive test of adherent sand grains and plant detritus. As it is figured with long arms and rather short tentacles its affinities are apparently with *Dendrosoma* rather than with *Lernæophrya*.

The genus *Trichophrya* seems also to be closely allied to *Dendrosoma*. It is found on the stalk of *Epistylis* and on the appendages of *Astacus* and various Entomostraca. We have found specimens which we attribute to the genus *Trichophrya* in our material obtained from the Bridge-

water canal. The genus may be distinguished from *Dendrosoma* by its small size, by the shortness and unbranched character of the processes that bear the suckers and by other characters which are described later (p. 174) (fig. 46).

The genus *Lernæophrya* was found by Perez (25) attached to *Cordylophora* in the docks at Bordeaux. We have also found it associated with *Cordylophora* in the material from the Bridgewater canal. It varies in size, but is usually larger than *Trichophrya*. It has short, unbranched arms and remarkably long suckers (fig. 48). In other respects also it appears to be distinct from *Dendrosoma* and other genera (see p. 176).

The genus *Dendrosomides* of Collin (17) was found on the thoracic appendages of *Eupagurus cuanensis*. It appears to have some affinities with *Dendrosoma*, but on the whole it seems to be more closely related to the *Ophryodendrinae*.

The genus *Staurophrya*,¹ Zacharias, is a free fresh-water form, with short arms, long non-capitate tentacles. Its affinities are obscure.

There is very little information at present as to the geographical distribution of *Dendrosoma*. All that can be said is that it has been found in Europe and in the United States of America. According to Ehrenberg it is found on *Ceratophyllum*, *Callitriche*, and on dead leaves. Kent found it on *Anacharis*, *Myriophyllum*, and other aquatic plants. Leidy (22) states that it is found in America on *Ceratophyllum*, and attached to the rotifer *Limnias socialis*. Stein (27) briefly refers to a species, *Dendrosoma astaci*, attached to the appendages of the fresh-water crayfish. As suggested by Kent it is very probable that this is the same species as *D. radians*.

¹ The only account of this genus we have found is in Delage's and Herouard's 'Protozoaires,' p. 514.

MATERIAL.

The first material we examined was supplied to us some years ago by Mr. Bolton, of Birmingham. Since that time he has forwarded to us living specimens on several occasions. The Birmingham material is usually attached to water plants.

A few years ago the Rev. T. Robinson, of Hale, informed us that *Dendrosoma* could be obtained in the Bridgewater canal, in the neighbourhood of Altrincham, attached to specimens of *Cordylophora*, and we have found it there and obtained an abundant supply at all times of the year. For many months it is principally attached to the hydrocauli of living *Cordylophora*, but we have also found it on the stalks of freshwater *Polyzoa* and on weeds. In the winter we have found specimens on the perisarc of the hydrocauli of *Cordylophoras* that have died down. It is noteworthy that we found these specimens a few days after the severe frost of December 25th to 30th, 1908. They were very plentiful, healthy, and were giving rise to a great many gemmulæ. They were also provided with a considerable number of epizoidic Urnulas. These facts seem to prove that there is no special advantage gained by the *Dendrosoma* in being associated with the *Cordylophora* other than that of position. It seems probable also that *Cordylophora* gains no advantage by the presence of *Dendrosoma* on its hydrocauli.

A difficulty in the investigation of the *Dendrosoma* found in the Bridgewater canal is, that the test becomes encrusted with a thick coat of what we can only call black dirt (text-figs. A and B). This renders the observation of the structure of the stolon and the proximal regions of the arms in anything but sections extremely difficult. The material obtained from Birmingham is much cleaner. Associated with the *Cordylophora* and *Dendrosoma* in the Bridgewater Canal there are several other genera of *Acinetaria*, *Vorticella*, *Carchesium*, *Epistylis*, and *Stentor*, besides worms, Rotifers et hoc genus omne.

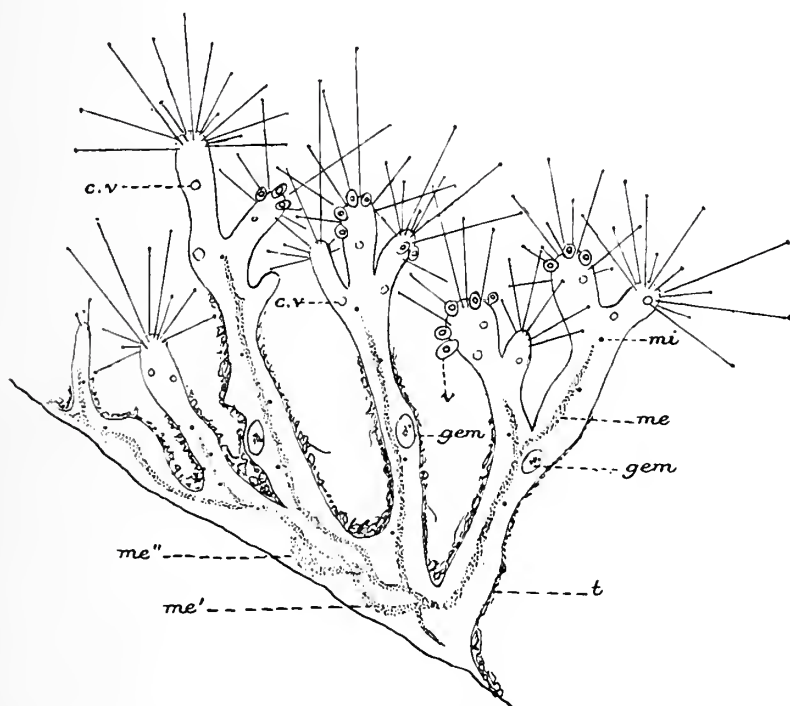
Of the other genera of these Acinetaria, two are of considerable importance in relation to *Dendrosoma*. These are the genera *Trichophrya* (Clap. and Lach.) and *Lernæophrya* (Perez).

At one period in our investigations we were inclined to believe that both these genera represent young phases in the life-history of, or varieties of, *Dendrosoma radians*, but we have since arrived at the definite conclusion that they are certainly distinct species. The relation between the three forms will be discussed later on, but we may state here that we are in agreement with Perez in considering *Lernæophrya* an intermediate form connecting *Trichophrya* with *Dendrosoma* and justifying the inclusion of the three genera in the distinct family the *Dendrosomina* (Bütschli). It is a matter of considerable interest that the three closely allied genera should be found associated together in this manner in the same locality. The struggle for existence between the different Acinetaria and Ciliata must be very keen, and it is surprising that the three genera should have survived side by side. In the material from Birmingham we found no examples of either *Trichophrya* or *Lernæophrya*.

The specimens from the Bridgewater canal consist of an irregular base or stolon attached to the perisarc of the host from which a number of free branches or "arms" project at various angles into the water (text-fig. A). It is difficult to form any very definite conception as to the size to which a single individual may attain, as the stolon is usually so thickly encrusted with dirt of various descriptions, and bends so frequently from one side of the *Cordylophora* to the other in its sinuous course, that the limits of the individual are often impossible to determine. We have measured single unbranched arms of this form that are .5 mm. in length (text-fig. B). The tentacles are very extensile, and may be 75-90 μ in length. The arms arise from the stolon at irregular intervals, sometimes in clusters of three or four, sometimes at intervals of one or two millimetres. There appears to be no geometric law governing the origin of the arms; but an arm

seems to be pushed out wherever an opportunity occurs for one to reach a food supply.

TEXT-FIG. A.

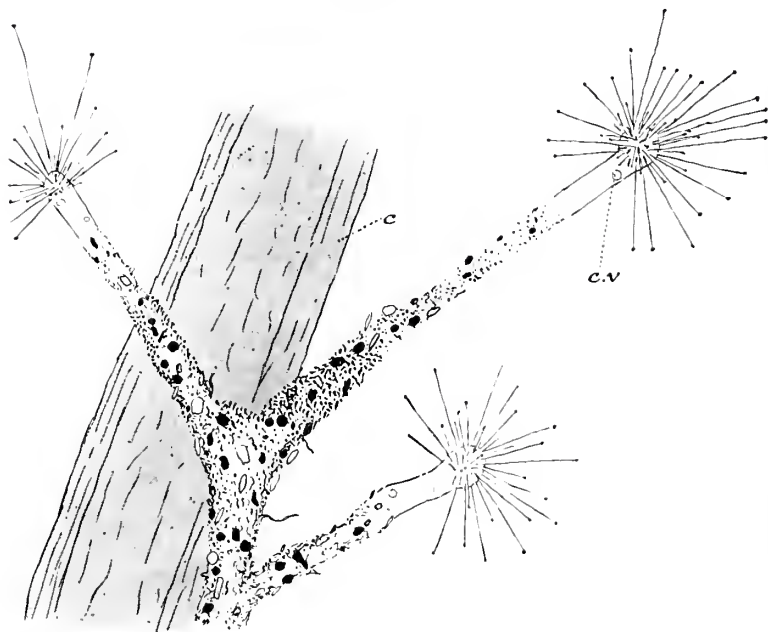


A specimen of *Dendrosoma radians*, attached to *Cordylophora*, from the Bridgewater canal, near Manchester. *c.v.* Contractile vacuoles. *gem.* The "internal buds" or gemmule. *me.* the long strap-shaped meganucleus. *me'* the meganucleus in the base of attachment, showing at *me''*, an irregular distribution of its chromatin granules in the cytoplasm; *t.* the test covered with dirt and foreign particles of various kinds. *v.* The so-called "external buds," now shown to be a species of *Urnula*, are seen at the extremity of some of the branches. The figure is semi-diagrammatic. The outline was drawn with the camera lucida from a very large, branched, living specimen, and the structural details filled in.

The specimens from Birmingham differ in some respects from those found in the Bridgewater canal. The former

attain to a greater size, their arms are longer and branch more frequently, and they seem to possess more micronuclei. It is difficult to express the differences between them in exact terms. Some specimens from Birmingham are very similar to some specimens from the Bridgewater canal, and there is

TEXT-FIG. B.



This figure represents the more usual form in which the variety of *Dendrosoma radians* from the Bridgewater canal is found. It is attached to the hydrocaulus of a *Cordylophora*. The longest of the three arms was 0.5 mm. in length and unbranched. The longest suckers are 75–90 μ in length.
c. *Cordylophora* hydrocaulus. *c.v.* Contractile vacuole.

undoubtedly a great range of possible variation in specimens from both localities. The specimens from Birmingham are undoubtedly more closely related to the original type specimens of Ehrenberg and to those described by Kent than are

the specimens from the Bridgewater canal, and we have no hesitation in naming them *Dendrosoma radians* Ehrenberg. It is a matter of opinion whether the specimens from the Bridgewater canal are sufficiently distinct to justify the constitution of a new species. After careful consideration we have come to the conclusion that they are not. All the principal differences, except that of the number of the micro-nuclei, may very probably be due to the direct influence of the environment.

Food.

For some time we were unable to record any observations on the food of *Dendrosoma*, the animal appearing to be very refractory in this respect as compared with *Dendrocometes*.

We have frequently observed small Ciliata and Flagellata swimming freely among the suckers, and even bumping up against them, without harm to themselves or producing any reaction in the suckers. In September of last year, however, we obtained in the plankton of some ponds near Manchester specimens of *Euplotes* and of a green *Paramecium bursaria*; and, on passing some of these under the coverslip of a preparation of living *Dendrosoma*, several were caught and held fast by the suckers, and the process of feeding began. The astonishing feature of the phenomenon is that *Dendrosoma* seems to prefer such large prey. A *Euplotes* 100 μ in length will be held and devoured by a *Dendrosoma* arm that is not more than 15 μ in diameter (fig. 30).

When an Infusorian is captured by a *Dendrosoma* it is not paralysed, but continues to lash its cilia with as great, or even greater, activity than before. It is almost painful to watch a *Euplotes* struggling to escape its doom. It will make a violent effort to move forward and then fall back exhausted for a few seconds, or it will endeavour to spin

round on its axis. But all the time particles of its protoplasm can be seen streaming down the suckers of the *Dendrosoma* into the arm. The *Dendrosoma* never seems to use the whole bunch of suckers at the end of an arm for feeding at the same time. From one to six or seven, according to the size and strength of the prey, may be used, the others being stretched out unconcerned with what is going on in their neighbourhood as if in search of other victims (fig. 30).

The usual statement made in the text-books is that the *Acinetaria* paralyse their prey before feeding on them, and this is used as an argument that a fluid of some kind passes down the tentacle towards the food before the return current of the food substance sets in. We are quite certain that in *Dendrocometes* such a paralysis of the food animals does take place. We have seen on several occasions small *Paramecia* caught and paralysed by the arms of this remarkable form. Bütschli (2) gives several examples of the paralysing properties of the *Acinetarian* suckers, and quotes cases observed by Stein in *Metacineta*, Maupas in *Sphærophrya*, Plate in *Hypocoma*.

Claparède and Lachmann (vol. ii, p. 50), however, record that a *Stylonychia* caught by "an *Acinetan*" struggled for a long time and then underwent fission. One of the daughter individuals escaped from the *Acinetan*, leaving her sister to be devoured.

Levick (23) also does not mention that the *Infusoria* upon which *Dendrosoma* feeds are paralysed, but says that the tentacles are capable of "resisting the struggles of the captive."

Since we made our first observations on the feeding of *Dendrosoma* we have occasionally found a smaller infusorian caught by the tentacles, but time after time we have failed to induce them to feed upon the common *Paramecium aurelia* from our cultures.

But although we have succeeded in inducing the *Dendrosoma* to feed we have failed to keep them alive in the laboratory for more than a few days. The material soon becomes

putrid owing to the death of the Cordylophora and the decay of the roots to which it is attached, and we have not succeeded so far in getting the specimens to become fixed to any other support.

THE STRUCTURE OF DENDROSOMA.

We have very little to add to the knowledge of the suckers and the general cytoplasm of *Dendrosoma*. The suckers are of considerable length, terminating in a small knob or cup. They are usually quite rigid when extended, like the suckers of most of the other *Acinetaria*, and they can be slowly shortened when circumstances become unfavourable. Being very slender and transparent we have not been able to demonstrate the presence of a definite lumen, nor have we seen even in the shortest suckers any evidence of a spiral ridge such as can be seen on the retracted suckers of some other *Acinetaria* (fig. 45). In several of our series of sections we have been able to trace very delicate lines running down some distance into the arm from the bases of the suckers.

The cytoplasm is usually clear and transparent. In the living arm a streaming movement of minute granules can be clearly seen, as originally described by Levick. In well-stained preparations the cytoplasm appears to consist of a delicate network of fibrils enclosing a number of minute granules which stain faintly with acid dyes, but there are very few elements in the cytoplasm that give a deep stain with basic dyes. We have found no evidence in our preparations of bodies corresponding to the "Tinctinkörper" of other *Acinetaria* in the arms or stolon where the meganucleus is clearly delimited. In some cases, however, when the meganucleus is scattered (see p. 156) the cytoplasm is filled with numerous chromatin bodies which may be derived from the meganucleus.

Martin (24) suggests in a recent paper that the "Tinctinkörper" of other *Acinetaria* represent in some cases the chromatin of the nucleus of ingested prey. The difficulty we

have found in getting *Dendrosoma* to feed may to some extent account for the absence of Tinctinkörper.

The contractile vacuoles occur at irregular intervals on the arms and may also occur in the stolons, but they are not easily seen except in those parts which are relatively free from the encrusting dirt (text-fig. A, *cv.*). The description of these structures given by Levick and Kent is correct; the elongated contractile vacuole figured by Claparède and Lachmann is obviously incorrect.

THE MEGANUCLEUS.

In a stained preparation of *Dendrosoma* the meganucleus usually appears as a dark band running along the axis of the arms and stolon. It is rarely exactly in the centre, but usually bends first to one side and then to the other, or runs a course for the whole length of an arm somewhat to one side of the exact axial line. In the younger parts of a stolon it has very much the same appearance as it has in an arm, but in the older parts it is frequently contorted, sometimes broken or discontinuous, branched, or knobbed, and not infrequently dissipated (figs. 11, 12, 13, and text-fig. A).

There is so much variation, however, in the general character of the meganucleus that it is impossible to describe it adequately in a single sentence.

Although it is usually found to some extent in every arm it never extends to the distal extremity as it is drawn in the figures given by Lang and Calkins, and in the younger and shorter branches there may be no part of the meganucleus at all (text-fig. A). We have observed a difference, which appears to be constant in this respect, between the specimens from the Bridgewater canal and those from Birmingham. In the former the meganucleus extends only a short distance into the arms, in the latter it extends very much further; but in this respect, as in others, the meganucleus is subject to considerable variation. In many specimens the limits of the meganucleus are very clearly defined, in others the boundary

lines are ill-defined and the difference between the meganucleus proper and scattered chromidia impossible to distinguish (figs. 12, 13, 14).

If the form and distribution of the meganucleus of *Dendrosoma* are remarkable, still more so is its minute structure. The difference between the meganucleus of *Dendrocometes* and that of *Dendrosoma* in this respect is very striking, and the first cause we had to doubt the current view that the so-called "external buds" (*Urnnla*) are really produced by the *Dendrosoma* was the difference we observed in histological detail between the meganucleus of these bodies and the meganucleus of the *Dendrosoma*.

We described in a former paper (16) the meganucleus of *Dendrocometes* as consisting of "a distinct meshwork of darkly staining lines which appears to support a series of minute, rounded, chromatin granules." "In the meshes of the darkly staining chromatin there is a homogeneous substance which stains faintly yellow with brazilin."

In the meganucleus of *Dendrosoma* we have found no trace of a true limiting membrane or of a meshwork. It consists simply of a number of chromatin granules floating independently in a fluid matrix (fig. 52). In this respect, therefore, the structure of the meganucleus appears to be very exceptional.

We have not arrived at these conclusions without very careful study and numerous experiments. We expected to find some kind of network, whether of plastin or of chromatin, such as we found in *Dendrocometes*, *Urnnla*, and some other *Acinetaria*, but the various methods we have employed have given us no positive results. Staining with hæmatoxylin and congo-red we have obtained very sharp differentiation of the histological structures, and in some sections we have seen a thin line bounding the meganucleus which might well be mistaken for a *membrana limitans*, but after prolonged research we are convinced that this line does not represent a continuous membrane, and that it belongs, not to the nucleus, but to the surrounding cytoplasm. Whether

our interpretation of this line is correct or not, the fact remains that there is a very marked difference between the megannucleus of *Dendrosoma* and that of other *Acinetaria* except *Lernæophrya* we have examined in this respect.

In referring to the megannucleus of *Acineta papillifera*, Martin (24) writes: "Generally in whole stained preparations numerous spherical dark areas are to be seen resembling the so-called "Binnen-körper" of the Infusoria. In section, these structures, as in the case of some Infusoria and *Dendrocometes*, are found to consist merely of local thickenings in the mesh of the nuclear network, and therefore resemble karyosomes rather than true nucleoli."

In *Lernæophrya*, so far as our observations go, the megannucleus resembles that of *Dendrosoma*.

In *Acineta tripharetrata*, according to Entz (13), the substance of the megannucleus appears to resemble that of *Dendrosoma* and contains a number of sharply defined bodies, but there is a distinct nuclear membrane.

Collin (7) describes the megannucleus of *Ephelota gemmipara* as consisting of "grains chromatiques de forme variée sur un substance achromatique," but he finds also a distinct isolable nuclear membrane.

In the iron-brazilin preparations a faint yellow colour can be seen in the thicker sections between the granules, and in iron-haematoxylin and congo-red preparations a faint pink colour may be seen in the same place, indicating perhaps that the matrix in which the chromatin granules float is to some extent capable of taking a faint oxychromatic stain, but no structure is seen in it even with the highest powers (Zeiss 2 mm.) of the microscope we have used in the best light.

There can be little doubt that the granules are mainly composed of chromatin. They give the characteristic stains with iron-haematoxylin, iron-brazilin, safranin, and thionin. They are usually spherical in shape, but occasionally irregular-shaped granules and large lumps (fig. 51) are found in the course of the band.

The variability in size may be seen by a comparison of figs.

50, 51, 52, which are drawn to the same scale. Fig. 50 represents the terminal extremity of the meganucleus in an arm, and the granules are of an average size. The diameter of the meganucleus in this specimen was $2\ \mu$, and the largest granules were approximately $0.85\ \mu$ in diameter. Fig. 51 represents a longitudinal section near the base of an arm in which there were two meganuclear bands nearly parallel with each other. The larger of these bands was about $3.4\ \mu$ in diameter. The granules in these bands are extremely small, but at intervals large lumps of chromatin, in some cases over $4\ \mu$ in length, occur. Fig. 52 represents a section through a remarkably swollen part of a meganucleus ($25.5\ \mu$ by $20.4\ \mu$), in which many of the granules are very large; the largest are about $2.5\ \mu$ in diameter. The study of the variation in size and form of the granules in the meganucleus suggests a comparison with the descriptions given of the meganucleus in *Carchesium* and other Ciliate Infusoria. In *Carchesium*, according to Mrs. Bidder (Greenwood, 14), there is a nuclear membrane which in certain stages is "demonstrable with difficulty"; the contents consist of granules of two kinds floating freely in the nucleochyme, and there is no indication of permanent linin threads connecting these granules together. The granules are of two kinds—the proto-macrosomes and the proto-microsomes, and of these the proto-macrosomes certainly and the proto-microsomes probably undergo changes in association with nutrition. The larger granules of the meganucleus of *Dendrosoma* seem to agree with the macrosomes of *Carchesium*, although we have not observed the vacuolated condition which the latter sometimes exhibit. A distinction between macrosomes and microsomes has not been demonstrated in *Dendrosoma*, but it is possible that further investigation might yield similar results to those obtained in *Carchesium*. This comparison suggests that the great difference we have recorded in the size of the granules of the meganucleus may be in some way associated with nutrition.

Division of the meganucleus.—The only method of

division we have been able to follow is the formation of the meganucleus or meganuclei of the gemmulæ. Before the outline of a young gemmula is seen in the cytoplasm, one or more knob-like outgrowths are formed from the ribbon-like meganucleus of an arm or of a part of the stolon. These grow, and as the outline of the gemmula is developed become constricted at the base, and ultimately the knob or knobs are separated off. The granules in the constricted part of the outgrowth become elongated, spindle-, or rod-shaped (fig. 20) during the constriction, but regain their spherical form as soon as the meganuclei of the gemmulæ are free. No distinction whatever can be drawn between the minute structure of the meganucleus where it is giving off these branches and that of other regions — in other words this division is purely amitotic. But it is not only amitotic, it is obviously unequal. In the amitotic division of the meganucleus of many Ciliata and of some Acinetaria (e. g. *Dendrocometes*) the division may be an equal division, the two products of fission in the former case, and the parent and gemmula in the latter may receive an equal portion of the parent meganuclear substance. In the case of *Dendrosoma* it is obvious that the gemmula receives only a small part of the total meganuclear substance of the parent. The meganucleus not only divides in gemmula formation but occasionally in ordinary vegetative growth, as is shown by the fact that the meganuclear bands are sometimes discontinuous. We have no reason to believe that this division is brought about by any other process than simple constriction.

The fragmented or disintegrated condition of the meganucleus may be seen in many preparations. It is by no means confined to the parts of the meganucleus in the stolon, but may occur in the basal portions of the arms. In some cases scattered chromatin granules (fig. 12) may be seen in the cytoplasm in regions where a distinct but not well-defined meganuclear band occurs, but in other regions the whole of the meganuclear structure appears to be scattered in the cytoplasm (fig. 13). If it is a fact that the mega-

nucleus under certain circumstances breaks up and is dissipated in the cytoplasm, we are reminded of the condition, long ago described by Gruber, of the meganuclear fragmentation in the ciliata, *Oxytricha* and *Lacrymaria*. An alternative hypothesis, however, might be put forward to the effect that the condition we have observed is not disintegration but construction of meganucleus; that the scattered granules we have observed were not cast out from the meganucleus, but are in process of formation in the cytoplasm, and will be added to the nuclear bands.

This alternative hypothesis will be considered more fully in a subsequent paper, but whatever hypothesis we adopt the study of the sections exhibiting this fragmented condition confirms most definitely the statements we have made above—that (1) there is no true nuclear membrane, and (2) that the granules of chromatin are not connected by a plastin network.

MICRONUCLEI.

The micronuclei of *Dendrosoma* have not been previously described. Their distribution in the arms and stolon varies a good deal, and it seems very probable that they are not constant in position but move about in the arms and stolon, driven hither and thither by the protoplasmic currents. On this point, however, we cannot speak with certainty as we have never been able to recognise the micronuclei in the adult living specimens. We have been able to distinguish them in some of the living gemmulæ.

They are not difficult to see in some of the ordinary stained whole mount preparations, although the crust of foreign bodies adherent to the pellicle sometimes interferes with the clear definition of the smaller internal structures.

The micronuclei may occur both in the arms and in the stolon. We have not observed them at any time very close to the distal extremity of the arms, although in one or two cases we have seen one or two micronuclei lying just beyond or close to the distal termination of the meganucleus (fig. 50, *m.*,

and text-fig. A, *mi.*). The arrangement of the micronuclei in the arms and stolon is very irregular. In some specimens they are found at fairly regular intervals of 30μ or 40μ in the arms, in others they seem to be in pairs (figs. 9, 10), indicating, perhaps, a recent epidemic of division, but in several specimens we have found clusters of three, four, five, or six micronuclei irregularly scattered in the arms and stolon. In the specimens from Birmingham we have noticed that, as a rule, more micronuclei are present than in the specimens from the Bridgewater canal. In the clusters there are sometimes eight or nine micronuclei together, and in one specimen there were as many as nineteen (fig. 15). This irregularity in the arrangement of the micronuclei has suggested the view that they may move about in the living organism.

In *Dendrocometes* and in other *Acinetaria* the micronuclei are usually surrounded by a clear area, the "hyaloplasm" of Maupas (fig. 14). Such a clear area is also seen round many of the micronuclei of *Dendrosoma*, but it does not appear to be so constant in character as it is in some of the other genera. In a former paper (16) it was suggested that this clear area may be due to a shrinkage of the micronucleus in preservation, and therefore of the nature of an artefact.

This view is confirmed by a careful examination with high powers of the area round the micronucleus of *Dendrosoma*, as it shows not the faintest trace of protoplasmic structure even in the most successfully stained preparations. Occasionally a few very delicate strands may be seen stretching across the area (fig. 14), indicating, perhaps, a former organic connection between the nucleoplasm and the surrounding cytoplasm. But in addition to the perfectly clear and unstainable area we can also distinguish a more irregular area of cytoplasm around each micronucleus, which is better defined in some cases than in others (fig. 13), that differs from the rest of the cytoplasm in being relatively free from granules and stains rather less deeply. It is this outer area which serves as a guide to the micronuclei and serves to distinguish them from other chromatin granules.

The minute structure of the resting micronuclei is very difficult to determine as they stain so deeply; but we have satisfied ourselves that there is a definite nuclear membrane and a network of chromatin (figs. 22, 23).

The resting micronuclei are always spherical in form. When seen in pairs and clusters they are about 3.3μ in diameter; when isolated they are sometimes as large as 4.5μ in diameter. Various stages in the division of the micronucleus have been observed, but others are missing. Our account of the process is not, therefore, quite complete. What we have seen may be regarded as a modified form of mitosis. It cannot be positively asserted that a more direct mode of division of the micronuclei does not take place, but as the different stages of division have been seen in areas where gemmule formation has begun, and also in areas where there is no evidence that gemmule formation is about to take place, and as, moreover, we have never seen any signs of direct division in the hundreds of micronuclei we have examined, it seems probable that amitotic division of the micronuclei never occurs.

The history of the mitosis so far as we can judge at present is as follows: The micronucleus swells and then becomes slightly oval in outline (fig. 24). The smallest specimen we have seen at this stage is 9.3μ by 5.1μ . It is difficult to determine whether the chromatin network has broken down at this stage or not, but definite lines can be distinguished running in the direction of the longer diameter. In the next stage the oval shape is changed to a spindle shape and the size is 15.3μ by 6.8μ (fig. 26). The chromatin seems to be withdrawn from the points of the spindle, which are usually quite clear at this stage. The chromatin is in the form of a large number of minute granules connected by a network of fibrils. It is possible that we have missed a stage here, as we have not found a spindle form yet with the chromatin collected together more definitely into an equatorial band.

We have found three or four examples of a stage in the

mitosis shown in fig. 25. In this stage the chromatin granules are arranged in lines running from the poles towards the equator, but there is a clear zone free from chromatin running through the equatorial belt. The three examples of micronuclei in this stage we have found measured 6.8μ by 6.3μ , 8.5μ by 5.6μ , and 6.8μ by 5.7μ respectively. They are oval or nearly spherical in shape. Being small and less pointed than the examples we have seen of the stage shown in fig. 26 it might be supposed that they come earlier in the mitosis. On the other hand, the separation of the chromosomes leaving a clear zone in the equator suggests that the metaphase has begun. If this is the correct interpretation of them then it seems probable that the onset of the metaphase is accompanied by a contraction of the figure.

In the next stage the chromatin granules are collected in two groups at the poles of the figure, which is 14.5μ by 4μ in size (fig. 27). In the broad band connecting these poles definite plastin lines can be distinctly seen. In the next stage we have observed the poles are further apart, the total length of the figure being 20.4μ (fig. 28). The poles are 5.1μ in diameter and contain an immense number of evenly scattered minute chromatin granules, but we have not been able to discern any plastin network connecting them. The band connecting the two poles has shrunk in the middle to 3.5μ in diameter. The same plastin lines may be seen in this band as in the last stage. In the final stage (fig. 29) the chromatin granules are more concentrated towards the inner hemisphere of each pole and there is an appearance of a "pole-plate" similar to that of *Paramecium*. The spindle is much narrower. Our examples of this stage are smaller than those of the last named, the measurements of the example figured being—total length 15.6μ , diameter of the poles 3.4μ , diameter of the spindle 1.7μ .

The history of the division of the micronuclei of *Dendrosoma* that we have just described is different in many respects from that we described in *Dendrocometes* in 1902; but

it must be remembered that in the case of Dendrocometes we described only the division of the micronuclei in conjugation. The mode of division of the micronuclei in gemmule-formation in Dendrometes differs in some respects from that seen in conjugation. On this subject we hope to write a further paper at a later date. We have not yet been fortunate enough to observe any phase in the conjugation of Dendrosoma.

It is possible that the minute granules of chromatin seen in various stages of the division of the micronucleus in Dendrosoma represent the chromosomes. With this view in mind we have carefully compared them in the earlier and later stages of the process to determine whether they are double in character before their separation in the two poles. In a recent paper Calkins and Cull (4) have shown that in the earlier maturation divisions of the micronuclei of *Paramecium aurelia* the chromosomes divide longitudinally. We can only state that we have not been able to find any evidence that the chromatin granules in these karyokinetic figures of Dendrosoma divide at all. Evidence of this, however, may be forthcoming from the stages we have missed.

THE GEMMULE.

The gemmulæ were first discovered by Levick, but it is difficult to understand from his figures what is their exact shape. If his fig. 4 is drawn accurately to scale the gemmule it represents was about $45\ \mu$ in diameter. Kent describes the gemmulæ as "hypotrichously ciliated embryos of relatively large size." Unfortunately he gives no statement of the size, but according to his figure they are about $33.3\ \mu$ in diameter.

Sand repeats Kent's statement that these bodies are hypotrichous, but adds that they are "en forme de lentille biconvexe aplatie," and have three contractile vacuoles (in correspondence with Kent's figure).

In the living material we have examined from the Bridge-

water canal two kinds of free-swimming gemmulæ were observed: a large kind which was plano-convex in side view (fig. 1), provided with a band of several rows of cilia and from six to ten contractile vacuoles; and a smaller kind (fig. 31), biconvex or loaf-shaped in side view, with an equatorial band of three or four rows of cilia and provided with only three or four contractile vacuoles.

In this material, however, we have found the *Acinetaria* attributed to the genera *Trichophrya* and *Lernæophrya* associated with *Dendrosoma*, and until recently we had no definite evidence as to which of the three forms the gemmulæ belonged.

In the material obtained from Birmingham no *Trichophrya* nor *Lernæophrya* forms were found, and the gemmulæ we found were all of the larger plano-convex type (fig. 1). This observation suggested that the larger type is the gemmula of *Dendrosoma*; but we obtained a definite proof of the truth of this suggestion by our observations on the development of two of these gemmulæ into the definite *Dendrosoma* forms as described below (p. 167).

We then examined carefully all our whole mount preparations for *Lernæophrya* forms showing gemmulæ in the brood-pouches, and found that the smallest of them was $24\ \mu$ in diameter, and the largest about $37\ \mu$ (the outline of this gemmula is indistinct and the measurement probably not quite accurate). The average diameter of eleven gemmulæ works out at about $29.6\ \mu$. The measurement of a larger number of gemmulæ of *Dendrosoma*, in their brood-pouches, gave us considerably greater diameters, but there are one or two complications that must be mentioned before giving the actual figures obtained. Whereas all the *Lernæophrya* gemmulæ were approximately circular in outline, many of the gemmulæ of *Dendrosoma*, in the aspect presented to us in the fixed preparations, were definitely oval in outline.

The smallest of the fully formed gemmulæ was $29.6\ \mu$ (circular in outline), the largest $40.7\ \mu$ by $55.5\ \mu$ (oval in outline). Many of the gemmulæ we measured were not fully

formed, and it may be that the size was not as great as it would be when the outline was completed. There is no reason to believe, however, that there is any considerable growth in the gemmula after the first faint lines marking out its contour are noticeable.

The average of the shorter diameters of sixteen gemmulæ was about 35.5μ and the average of the longer diameters about 41μ . We may roughly express, therefore, the difference in size between the two kinds of gemmulæ as a difference of 29.6μ by 29.6μ in *Lernæophrya* and 35.5μ by 41μ in *Dendrosoma*. There is not much difference in size, probably, between the largest gemmulæ of *Lernæophrya* and the smallest gemmulæ of *Dendrosoma*, and there is clearly considerable variation in the size of the gemmulæ of both genera.

But still it may be considered a fact of some systematic value that the gemmulæ of *Dendrosoma* are larger than those of *Lernæophrya*. We found, unfortunately, no *Trichophrya* forms showing formed gemmulæ, and we can therefore give no near details of them. They have been described by Bütschli, Sand, and others, but we have not found any measurements given of them in the literature of the genus.

The gemmulæ of *Dendrosoma* are usually found in well-developed individuals, and are frequently situated at the base of the arms (text-fig. A), but they also occur in the course of the arms and more rarely in the stolons.

The gemmulæ at the base of the arms is a very characteristic feature of the specimens from the Bridgewater canal. In the specimens from Birmingham the gemmulæ are more frequently found some little distance above the base.

In the earliest stage of gemmule formation there is a slight swelling in the arm, and two or three curved lines (fig. 19, *o.*) appear in the cytoplasm and mark the external boundary of the future gemmula. A narrow crescentic space often appears between these lines and the cytoplasm. The meganucleus sends off a short branch which ends in a knob-like swelling towards the concavity of these lines (fig. 20). The

base of this arm of the meganucleus gradually becomes constricted, and at a later stage divides, leaving the knob-like extremity as the meganucleus of the gemmula (conf. p. 156).

In several cases, particularly in specimens from Birmingham, we have observed two of these processes entering into the gemmula area, and we have several preparations of young gemmulae with two distinct meganuclei (figs. 16 and 21). When there are two meganuclei in one gemmula there seems to be a great deal more meganuclear substance in proportion to the cytoplasmic substance than when there is only one meganucleus. A comparison of the different gemmulae with only one meganucleus renders it difficult to believe that there is any definite relation between the cytoplasm and nucleoplasm in gemmule formation.

One or two, or possibly in some cases more than two micronuclei in the vicinity of this swelling increase in size until they are about $11\ \mu$ in diameter. One, or possibly more than one of them, becomes spindle-shaped, attaining to a size of nearly $14\ \mu$ in length by $11\ \mu$ in greatest diameter (fig. 19). This micronucleus then divides by mitosis (see p. 159).

While the micronucleus is thus dividing the boundary lines of the gemmula are in the process of completing the enclosure of the gemmule area. The division of the micronucleus is, however, completed, and the daughter-nuclei have considerably shrunk some time before the area of the gemmule is entirely delimited from the protoplasm of the arm.

It is not possible for us to state definitely that there is any constancy in the number of micronuclei taking part in the bud formation.

In the bud that is still in the brood-pouch, shown in fig. 17, there is only one micronucleus. In the specimen shown in fig. 19 two micronuclei are taking part in the formation of the gemmula; in the young form shown in fig. 18 there are three micronuclei (only two are drawn, the third being hidden by the meganucleus); in that shown in fig. 21 there are four, and in that shown in fig. 16 there are seven. We have never

seen any evidence of division of the micronucleus during the free-swimming gemmula stage nor in younger fixed stages. It seems probable, therefore that the number of micronuclei taking part in the formation of the bud may vary from one to seven. In *Dendrocometes* we also found that the number is not constant, but varies from two to five.

In *Dendrosoma* then, as in *Dendrocometes*, the meganucleus of the gemmula is formed by amitotic division of the meganucleus of the parent, and the micronuclei of the gemmula are formed by mitotic division of the micronuclei of the parent.

THE FREE-SWIMMING GEMMULA.

We have called attention to the fact that there is considerable variability in the size of the gemmulae of *Dendrosoma*. The gemmula shown in fig. 1 was plano-convex in form, with a broad girdle of several rows of cilia extending from the middle almost to the edge of the plane surface. It was 60μ in diameter and 40μ in height. It was difficult to count the number of contractile vacuoles or to be certain they were constant in number, but there were certainly more than three, and probably from eight to ten. Their rhythmic contractions were not synchronous, and frequently, but not constantly, two or three vacuoles in close contact made their appearance when previously only one was seen.

The meganucleus in this form could be clearly seen in the centre of the protoplasm when the gemmula was viewed from above or below. It was sometimes spherical, but varied in shape, and frequently showed one or two lobate processes like the pseudopodia of an amoeba.

This particular gemmula was kept under observation for two days, and gave rise to a young suctorian, which was clearly a *Dendrosoma radians*.

The free-swimming gemmulae of the second kind (p. 161), found in the material from the Bridgewater canal, are probably the gemmulae of the *Lernaeophrya* forms. They have

some resemblance to the figures and description of the gemmula of *Trichophrya epistylidis* given by Bütschli (2, Pl. 78, 6b).

According to Sand, however, the reproduction of *Trichophrya epistylidis* is "par embryons internes multiples (tous situés dans la même cavité), en forme de lentille biconvexe, munis de 3 couronnés de cils et de 3-8 vacuoles contractiles."

According to Stein the four to eight gemmulæ that may be found in the brood-pouch of this species are produced by the fission of a single gemmula.

We have not observed more than a single gemmula in any brood-chamber of either *Lernæophrya* or *Dendrosoma*, but we have not seen enough specimens of *Trichophrya* yet either to affirm or deny Sand's statement that the gemmulæ are multiple in that genus.

The gemmule formation in the specimens of *Lernæophrya* resembles that described by Perez (25) for the specimens obtained by him at Bordeaux in the curious fact that it sometimes occurs very early. In our figure (32), for example, we have drawn an example of a very young *Lernæophrya* (or *Trichophrya*?) which has only just settled down, but already shows a fully formed gemmula in the brood-pouch.

We cannot give a decided opinion as to whether all the free-swimming gemmulæ of this biconvex type belong to *Lernæophrya* or to *Trichophrya*, as we have not at present been able to trace out their history after they have settled down, but it is almost certain that they belong either to the one genus or the other, and not to *Dendrosoma* or any of the other *Acinetaria* associated with it.

These gemmulæ are usually biconvex, with a slight constriction in the middle, an equatorial band of four rows of cilia, and three contractile vacuoles (fig. 31). There is probably a considerable range of variation (see p. 162) in size and shape. One of the convex sides is sometimes rather more flattened than the other; there may be only three, or possibly more than four, rows of cilia in the equatorial band (the exact

number of rows being difficult to determine unless the gemmula is seen sideways and the cilia are moving slowly). The number of contractile vacuoles may in some cases be two or four, but is usually three.

A remarkable point in the structure of these gemmulæ is that at present we have no definite evidence of the presence of micronuclei. Neither in the free-swimming gemmulæ themselves nor in the preparations (both whole mounts or sections) of the gemmulæ in the brood-chambers, nor in the body of the *Lernæophryas* and *Trichophryas* have we been able to find the micronuclei in any form.

Certain deeply stained granules are sometimes found scattered in the protoplasm, and some of these may be, and probably are, micronuclei. It is very improbable that these or any other *Acinetaria* are not heterokaryote, but we imagine that in *Lernæophrya*, and possibly in *Trichophrya* too, the micronuclei are very small or involved with the meganuclei.

Further investigation is needful before the mystery of the micronuclei of these forms is solved.

DEVELOPMENT OF THE GEMMULA.

We have observed the development of the gemmula in the Birmingham material twice. The history of one of these, September 15th, 1908, was as follows:

A free-swimming gemmula settled down on the cover-glass between 11.45 and 12 noon. The cilia disappeared entirely in forty minutes, and as they disappeared short suckers were developed all over the body, except, of course, the part attached to the cover-slip. As to the method of disappearance of the cilia we have nothing but the negative evidence to offer that we have not seen them break off. The probability is that they are withdrawn. We feel certain that they are not converted into suckers. At 12.30 the embryo was in a similar stage to that represented by fig. 3, and closely resembles the stage figured by Bütschli for *Trichophrya* (2, Pl. 78, 6 c.).

It was slightly oval in outline, the two diameters being $55.5\ \mu$ by $51.8\ \mu$. A preserved and stained gemmula of this stage is shown in fig. 18. At 1.30 the tentacles had considerably increased in number and in length on one quadrant of the gemmula, but had diminished in number over the rest of its surface. At 2.10 the main body of the gemmula had increased to $62\ \mu$ in diameter, but on one side a short arm process ($7.2\ \mu$), supporting a great many tentacles, had been protruded. There was only one tentacle left on the main body. Six contractile vacuoles were observed at this stage, and the meganucleus was visible and had an amoeboid form with two short, thick, pseudopodia-like processes. At 3.10 the arm was $25.9\ \mu$ in length and the tentacles confined to its distal extremity. For the first time a single contractile vacuole was seen at the base of the arm. At 3.55 the arm had increased to $33.3\ \mu$ in length, and a new arm at right angles to it was beginning to be formed. At 4.40 the main body was losing its circular outline. The new arm, about $14.8\ \mu$ in length, exhibited tentacles, and the first formed arm was $48.1\ \mu$ in length. Three micronuclei were clearly visible at this stage in the clear protoplasm. The meganucleus had four branches, one directed towards the longer arm, but not extending into it, one towards the shorter arm. Another specimen of a *Dendrosoma*, which was probably of the same age as this one, was found in a whole mount preparation and is shown in fig. 21. The meganucleus is in two parts, but the general form of it, apart from this peculiarity, is very characteristic of young *Dendrosomas* of this stage. In this particular specimen there were four well-developed micronuclei.

The other case of the development of a young *Dendrosoma* from a free-swimming gemmula is illustrated by the figures 2-7. The ciliated gemmule, at the time it settled down, had from eight to twelve contractile vacuoles. The meganucleus could be clearly seen, as it was quite colourless as compared with the pale yellow tint of the surrounding cytoplasm. It was distinctly amoeboid, constantly, but slowly, changing its shape (fig. 2). Before the cilia disappeared numerous

short suckers were produced, scattered irregularly but principally near the margin of the body. The body of this specimen did not retain its circular form, but became irregularly quadrilateral. After a period of two hours (fig. 4) the cilia had disappeared and the suckers were mainly collected at the two ends, but two or three odd suckers were observed in the middle. In another hour there were three distinct tufts of suckers (fig. 5), but two hours later still all the suckers had disappeared except one isolated one, and a dense tuft at one end (fig. 6). The next morning, i. e. eighteen hours later, the end supporting the tuft of suckers had grown considerably to form a definite *Dendrosoma* arm (fig. 7).

The stage just described corresponds with that figured by Savile Kent (in his Pl. 47, fig. 21), but differs from it in the absence of suckers on the general surface of the body. We have seen a good many specimens of this stage but never one which, possessing a well-defined arm, had suckers scattered over the rest of the body, as shown in Kent's figure. In Kent's figure of this stage only three contractile vacuoles are shown. Levick, however, gave another figure in which six contractile vacuoles were shown. The latter is in this respect, as well as in the actual size, more in accordance with our observations than the former.

The so-called "external buds."—In the literature of *Dendrosoma* reference is made by nearly all authors to another method of reproduction than that by the gemmulae previously described. Kent observed at or near the distal extremity of the arms of many specimens a number of spherical or oval bodies, which he believed to be "exogenously produced germs similar to those of *Acineta mystacina* of Stein." It is probable that these are the same bodies as those previously described by Levick as "ovaries." Bütschli describes them as "angebliche freie äussere Knospen," and Sand as "gemmes externes ciliées quelquefois tentaculées, produites à l'extrémité des rameaux."

We have found bodies similar in position, form, and size to these so-called external buds in our specimens from the

Bridgewater canal and from Birmingham, and we have discovered that they are epizoid Acinetarians belonging to the species *Urnulla epistylidis*.

The possibility that the bodies described and figured by Kent are different from those we have observed has of course occurred to us. It is, however, very improbable that external buds could be formed in the position of these bodies for the following reasons :

In the formation of a bud it would be necessary for the meganucleus to take part. The meganucleus of *Dendrosoma*, however, does not extend as far as the extremity of the branches, and could not possibly take part in the formation of buds in the position assigned to the "external buds" by Saville Kent.

We have examined a great number of specimens of *Dendrosoma* from the two localities, obtained at different seasons of the year and in varying phases of activity, but we have never seen any reproductive bodies of the form and in the position assigned to the "external buds"; but *Urnulla epistylidis* does occur in this position in a large majority of the specimens examined, and frequently in considerable numbers.

ON URNULLA EPISTYLIDIS.

This interesting species was first described by Claparède and Lachmann (5). It is mentioned in their first volume (1858-9), but the reproduction is more fully described in the second volume (1860-61). It was found on the stalk of an *Epistylis*. Owing to the appearance of a branching tentacle in one of their specimens these authors regarded *Urnulla* as a Rhizopod and placed it next to the genus *Euglypha* in the family *Actinophryina*.

Engelmann (12) and subsequent authors have, however, agreed that it is an Acinetarian, and Bütschli places it in the family *Urnullina* with the genera *Rhynceta* and *Acinetopsis*—an arrangement that is followed by Sand.

Only one species has been described—*Urnulla epistylidis*

—and the measurements given by Sand of this species are : Length of test, 20μ – 120μ ; diameter of test and body, 15μ – 80μ ; diameter of the nucleus, 5μ – 15μ . The genus is characterised by the definite but very thin test, which is usually conical in shape and curved proximally towards the disc of attachment. There are one, or two (rarely more than two) tentacles. The reproduction is by oblique unequal fission, and the smaller of the two products of fission escapes as a ciliate gemmula.

The question whether the species of *Urnula* we have found on *Dendrosoma* should be referred to the species *U. epistylidis* or placed in a new species may be open to discussion; but we can find no satisfactory reasons at present for adopting the latter course.

The specimens attributed to *U. epistylidis* by Sand and other authors are very variable in size (15μ – 80μ in body diameter), so that the fact that the largest of the specimens we have measured is not more than 30μ in this diameter and the average is about 25μ does not signify more than that the *Urnula* on *Dendrosoma* belongs to a small race.

In the original figure given by Claparède and Lachmann the two tentacles are shown to arise from the side of the body turned towards the host. In most of our specimens the tentacles arise from the distal surface or apex, as shown in figs. 35–39. The original figure with the tentacles arising from the sides has been copied in the subsequent papers and books, and it seems to be by no means certain that this origin is normal in the species. The body may rotate more or less in the test and the appearance shown may be only temporary, but we have observed only one or two cases in which the origin of the tentacles may have been lateral.

The position of the contractile vacuole was not constant in our specimens. It is usually situated, as shown in figs. 35–39, near the centre of the distal convexity, but in several specimens we have seen it more deep-seated. In the figures of the species given by other authors it is shown by the outer side of the nucleus. The structure of the test, which is extremely delicate, appears to be the same as that previously described,

except that we have observed a slight disc-like swelling at the point of attachment.

After full consideration of all these points of apparent differences, and bearing in mind the possibility of considerable variation in adaptation to the conditions, we have come to the conclusion that the *Urnula* found on *Dendrosoma* should be referred to the species *U. epistylidis* (Claparède and Lachmann).

It is not necessary to give a full description of the species, but we will be content with a few remarks on some special characters. The tentacles are the most remarkable features. In the first place they are comparatively rarely seen, the body of the *Urnula* being usually rounded off within the test and at rest. In the majority of specimens which do exhibit tentacles at all only one is seen. When there are two they usually cross one another as shown in fig. 39.

It is extremely probable that a specimen that at one time exhibits only one tentacle may at another time exhibit two, or even three, tentacles. The tentacles of *Urnula* differ from those of the typical *Acinetaria* in two respects. They are relatively very long and flexible, moving actively with curious serpentine curves as if in search of food. They do not terminate in a sucker.

When fully extended they are very delicate and attenuate at the distal extremity to a very fine point (fig. 35). When partially retracted or not fully extended they are much thicker, show a spiral marking (fig. 45), and terminate in a spindle-shaped, or sometimes bluntly club-shaped, extremity. There can be no doubt that they are protruded and withdrawn into the body with considerable rapidity.

We have not been able to satisfy ourselves as to the food of *Urnula*. We have frequently observed a tentacle bent over towards the head of the *Dendrosoma*, with its pointed end buried among the bases of the *Dendrosoma* suckers. This attitude suggested that the *Urnula* is parasitic on the *Dendrosoma*, and this suggestion is confirmed by the fact that the heads of *Dendrosoma* affected by *Urnula* do not look

so healthy as those that are free from them. On the other hand, healthy Urnulas are frequently found on *Dendrosoma* itself in positions that would not permit them to penetrate the delicate unprotected ectosarc of the head region, and also on other bodies, such as weeds, stalks of *Epistylis*, etc. We have, moreover, never observed a stream of food particles passing from the *Dendrosoma* to the Urnula body in the tentacle that is apparently attached to the former, as we should certainly find if the latter were feeding parasitically upon it. We are inclined to the opinion, therefore, that the Urnula is epizoid and not strictly parasitic.

The meganucleus is usually spherical in shape and central in position. In one specimen (fig. 40) in which the diameter of the body as preserved was $19\ \mu$, the diameter of the meganucleus was $8.5\ \mu$. The chromatin of the meganucleus is usually in the form of a network. It never shows the granular character that is such a marked feature of the meganucleus of *Dendrosoma*. It is sometimes difficult to determine whether a micronucleus is present or not, but a small deeply staining granule about $1.7\ \mu$ in diameter may frequently be observed in sections which we believe to be the micronucleus (fig. 40). No stages in its enlargement or division have been observed.

We have observed the same method of reproduction in our specimens as that previously described for the species by Claparède and Lachmann. The individual divides by oblique fission into two parts, one usually larger than the other (figs. 41, 43). Of these the smaller becomes holotrichously ciliated and escapes. The larger may remain in the undivided lorica and increase in size until it is again full grown. Of this, however, we have no positive evidence. It is possible, however, that the escape of the smaller product of fission entails the death of the larger product, but if this were the case we should expect to find attached to the *Dendrosoma* a certain number of empty loricae. We have, however, never found an empty lorica attached to the *Dendrosoma* nor any signs of degenerating protoplasm in a lorica.

The Urnula was found on specimens of *Dendrosoma* from

both localities. Sometimes a few specimens may be found that are quite free from these epizotes, but it is very rarely the case that a single collection of *Dendrosoma* is brought in that does not show some infected specimens. The number varies a good deal, but there is no reason to believe that they are more numerous at one season of the year than at another.

The settlement of the gemmula and the development of the lorica have been observed by one of us (W.) on two or three occasions. The free-swimming holotrichously ciliated gemmula is about 20μ by 15μ in size. The cilia stop moving and begin to disappear about ten minutes after settlement on the *Dendrosoma* is effected. The lorica must be formed very rapidly as the protoplasm is contracted into an oval form near its free end, as in fig. 35, about five minutes after the settlement. The cilia are still plainly visible, but in another five or ten minutes they disappear. A single tentacle begins to grow out a few minutes after the cilia have disappeared.

A curious feature that was observed on both occasions was the presence of two or three minute capitate tentacles at the time the cilia are disappearing. They are, however, only present for a few minutes, and cannot be recognised at all when the characteristic *Urnula* tentacle is developed.

In the figure given by Saville Kent one of the supposed "external buds" is drawn with six short capitate tentacles. It is possible that Kent may have observed an *Urnula* that had just settled down and still retained the temporary capitate tentacles we have described.

FURTHER REMARKS ON THE SYSTEMATIC POSITION OF *DENDROSOMA*, *LERNEOPHYA* AND *TRICHOPHYA*.

The relation of these three genera has already been briefly referred to in the introduction, but a further summary of the characters that distinguish them may be useful in the light of the observations we have recorded in this paper.

The genus *Trichophrya* was described by Claparède and Lachmann, 1858-61. The original type-species is *T. epistylidis*, a common species usually attached to the stalk of *Epistylis*. We have found it frequently in the Bridgewater canal collections, and the specimen we have drawn in fig. 46 *Tr.* was about 129.5μ by 111μ in size.

Taking this as a type we may say that it differs from a full-grown *Dendrosoma* in its small size and the relative shortness of its arms. It might be thought to be a young *Dendrosoma*, but it differs from the young *Dendrosoma* in having several short arms instead of only one or two. The young *Dendrosomas* shown in figs. 47 and 48 are smaller than the *Trichophrya* shown in fig. 46, but nevertheless exhibit the characteristic *Dendrosoma* form.

There must also be some important difference between them in the character of the micro-nucleus, but the nature of this difference we cannot describe. It is perfectly easy, as our figures show, to demonstrate the presence of micro-nuclei in young *Dendrosoma*, but we have not yet been able to find definitely the micronucleus in any specimen of *Trichophrya* we have examined.

In *Trichophrya epistylidis*, according to Stein, Bütschli, Sand, and other writers, the single gemmula that is formed in the brood-chamber may divide into four or eight gemmulae before liberation. We have not observed a similar mode of reproduction either in *Lernæophrya* or in *Dendrosoma*.

The figure given by Bütschli of the free-swimming gemmula of *T. epistylidis* shows that it must be very similar in shape to the gemmula we have ascribed to *Lernæophrya* (fig. 31).

Several other species of the genus have been described by Sand and others, but of these we have very little detailed information. Some of them, such as *T. salparum*, *T. amœboides*, *T. odontophora* and *T. mirabilis* are marine. One of these at least, *T. mirabilis*, found attached to hydroids at Banyuls, may possibly be more closely related to the genus

Lernæophrya as it is characterised by its very long suckers.

The genus *Lernæophrya* was described in 1903 by Perez (25). We have found it in the Bridgewater canal, and, like the Bordeaux type-specimens, attached to *Cordylophora*.

Lernæophrya is a larger form than *Trichophrya*. According to Perez it may attain to a size of 400μ – 500μ . Our specimens are not as large as this, but we have found them over 200μ in length (fig. 49).

They differ from both *Trichophrya* and *Dendrosoma* in the extraordinary length of the suckers. Perez says he has measured suckers 400μ in length. Our specimens were smaller than his, but we have found some of the suckers to be over 275μ in length. Perez states that the gemmulae are frequently formed at a very early stage, before the arms are formed. We have found the same peculiarity in some young forms which we attribute to *Lernæophrya*. In our fig. 32 we have drawn a young Acinetarian, which is probably a young *Lernæophrya*, although we have no conclusive evidence to prove that it is so, in which there are no arms and only four suckers, but it nevertheless contains a full-grown gemmula in its brood pouch.

As in *Trichophrya* so in *Lernæophrya* the micronuclei have at present escaped our observation, and as Perez does not mention these structures in the description of his specimens it is possible that some peculiarity of the micronuclei, which renders them obscure in the resting stage, is a character which *Lernæophrya* and *Trichophrya* have in common.

Only the one species, *L. capitata* Perez, has at present been described. Our specimens do not appear to differ from the type except in size, and we are inclined, therefore, to regard them as a small race of the type-species.

The gemmulae described by Perez were 50μ in diameter, whereas the largest we have measured were only 37μ in diameter, but in other respects they seem to agree.

The important characters in which the genus *Dendro-*

soma differs from *Trichophrya* and *Lernæophrya* are its greater size, the greater length of its arms, and the characters of the gemmula. The suckers of *Dendrosoma* vary a great deal in length, according to circumstances, but they never attain to the same actual or relative length as the suckers of *Lernæophrya*. The free-swimming gemmulæ of *Dendrosoma* differ from the gemmulæ which we attribute to *Lernæophrya* in size, in shape, in having several instead of only three or four bands of cilia, and in having several contractile vacuoles instead of only three.

A prolonged study of the specimens of *Dendrosoma* from the Bridgewater canal and from Birmingham give some grounds for the view that they belong to different species.

These differences have been previously mentioned (p. 147); it is only necessary in this place to refer again to the difference in the number of the micronuclei. In looking through a number of preparations of specimens from the two localities, the large number of the micronuclei in the Birmingham specimens is often a very striking feature. To take two extreme cases, the piece of an arm that is drawn in fig. 15 showing nineteen micronuclei in a cluster round the meganucleus, and a gemmula showing seven micronuclei, such as that drawn in fig. 16, we should recognise at once as belonging almost certainly to the Birmingham variety. On the other hand, when the micronuclei are isolated or in pairs at considerable distances apart, as shown in text-fig. 1 and in figs. 9 and 10, there would be a strong probability that they were taken from specimens of the Bridgewater canal variety.

But nevertheless specimens of the canal variety are sometimes found in which several micronuclei are aggregated together, as shown, for instance, in fig. 8, where six micronuclei form a cluster, and in many specimens from Birmingham the micronuclei are scattered in very much the same way as in the canal variety.

To endeavour, therefore, to make a specific character of the number of the micronuclei, a character which is obviously subject to great variation, would be a task of great difficulty

and very little practical value. Joseph (18) has shown that there is a great range of variation in the number of micronuclei in the Ciliate *Loxodes*, and it is clear from this and from other evidence that it is not safe to base specific differences on the number of the micronuclei.

SUMMARY OF RESULTS.

Specimens of *Dendrosoma* were found in the Bridgewater canal attached to *Cordylophora*, *Polyzoa*, and weeds. They differ in some respects from the type of *Dendrosoma radians*, and may be regarded as constituting a distinct race.

The meganucleus does not extend to the extremity of the arms as previously described and figured. It has no true nuclear membrane and no linin or plastin supporting network, but consists of numerous chromatin granules, "chromidia," floating freely in a nuclear sap.

There are numerous micronuclei, usually about $4\ \mu$ in diameter, which divide by mitosis. Reproduction is effected by the formation of internal buds, "gemmulae." They are plano-convex in form, $35.5 \times 41\ \mu$ in diameter, have several contractile vacuoles and a broad band of cilia. The description of these gemmulae given in this paper differs in some respects from that of previous authors.

The gemmulae of *Lernaeophrya* are also described.

The development of the gemmulae of *Dendrosoma* is described.

The "external buds" of Saville Kent are proved to be epizoic *Acinetaria* belonging to the species *Urnulla epistylidis*.

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EXPLANATION OF PLATE X,

Illustrating the memoir by Messrs. Hickson and Wadsworth
on *Dendrosoma radians*.

LETTERING.

A. Arms of *Dendrosoma*. *chr.* Chromatin granules. *c.v.* Contractile vacuoles. *D.* Swollen end of the arm of *Dendrosoma* in fig. 30. *E.* Euplotes. *Ep.* Epistylis. *g.* Gemmula in brood-pouch. *M.* Megannucleus. *M.g.* Megannucleus of gemmula. *m.* Micronucleus. *O.* Outline of gemmula in fig. 19. *s.* Sucker. *st.* Stream of food-particles. *t.* Tentacle of *Urnula*. *Tr.* Trichophrya in fig. 45.

Figs. 1-7.—Illustrating the free-swimming gemmula of *Dendrosoma* and its development after fixation.

Fig. 1.—Side view of the free-swimming gemmula, showing the band of several rows of cilia and four of the peripheral contractile vacuoles. $\times 250$.

Fig. 2.—Surface view of a gemmula immediately after fixation. The megannucleus (*M.*) has an amœboid form.

Fig. 3.—Gemmula as seen about thirty minutes after fixation, showing the suckers (*s.*) that have begun to sprout out from the general surface. Cilia are still present but comparatively few in number.

Fig. 4.—Young *Dendrosoma* two hours after fixation.

Fig. 5.—Young *Dendrosoma* three hours after fixation.

Fig. 6.—Young *Dendrosoma* five hours after fixation.

Fig. 7.—Young *Dendrosoma* one day after fixation. All the suckers are now confined to the extremity of the single arm.

Fig. 8.—Section through a part of the stolon of a *Dendrosoma* showing a cluster of six micronuclei (*m.*).

Fig. 9.—Section through an arm on the same slide showing the micronuclei in pairs. Each of these micronuclei are about $3.5\ \mu$ in diameter.

Fig. 10.—Section through another arm in the same preparation showing another pair of micronuclei and a contractile vacuole.

Fig. 11.—Section through a portion of a stolon showing a double and contorted meganucleus.

Fig. 12.—Section through a portion of an arm showing chromatin grains scattered in the cytoplasm. *M.*, the main meganucleus.

Fig. 13.—Section through another part of an arm showing a single micronucleus (*m.*) about $4\ \mu$ in diameter and the fragmented meganucleus.

Fig. 14.—Section through another arm more highly magnified to show the structure of the cytoplasm and nuclei. The micronucleus is $3.2\ \mu$ in diameter, the largest chromatin granules in the meganucleus about $1.5\ \mu$ in diameter.

Fig. 15.—Drawing of a part of the arm of a whole-mount preparation of a specimen of *Dendrosoma* from Birmingham showing a cluster of nineteen micronuclei.

Fig. 16.—Section through a gemmula of *Dendrosoma* from Birmingham showing two distinct meganuclei (*Mg.*) and seven micronuclei (*m.*). The diameters of this gemmula were $55\ \mu \times 48\ \mu$.

Fig. 17.—Transverse section through an arm showing a newly formed gemmula in position. The meganucleus of both the gemmula and of the arm have discharged some chromatin grains (*chr.*) into the general cytoplasm. The diameter of the bud is $22\ \mu$, of the micronuclei about $5.5\ \mu$.

Fig. 18.—A gemmula soon after it has become fixed from a stained preparation, showing the band of cilia (*c.*) and two suckers (*s.*). The diameters of the gemmula are $38\ \mu \times 37\ \mu$ and of the micronuclei $5\ \mu$ and $48\ \mu$.

Fig. 19.—An oblique section through an arm of *Dendrosoma* showing an early stage in the formation of a gemmula. At *o.* are shown the curved lines that mark the boundary of the gemmula. Two micronuclei of the arm have enlarged previous to division. The sizes of the micronuclei (*m.*) in this preparation were $15.3\ \mu \times 6.8\ \mu$ and $9.3\ \mu \times 5.1\ \mu$ respectively.

Fig. 20.—Transverse section through an arm showing the outline of a gemmula and the method by which a part of the meganucleus of the arm is pinched off to form the meganucleus of the gemmula. In this preparation some of the contractile vacuoles could be seen. No micronuclei were observed in this section. The diameter of the gemmula from *a-b* was $37.4\ \mu$.

Fig. 21.—Young *Dendrosoma* observed alive from free-swimming gemmula stage (September, 1908) and then fixed. It is $85\ \mu \times 70\ \mu$ in size, the arms about $20\ \mu$ in length, and the four micronuclei each about $4\ \mu$ in diameter.

Figs. 22–29.—Series of stages seen in the mitotic division of the micronuclei. $\times 1000$.

Fig. 22.—A full-sized micronucleus in a resting condition.

Fig. 23.—Enlarged micronucleus previous to mitosis.

Fig. 24.—Micronucleus in stage of spindle-formation.

Fig. 25.—Stage of division in which there is an equatorial band free from chromatin.

Fig. 26.—A stage in mitosis occasionally seen in which the poles are pointed and free from chromatin. The relation of this stage to the other stage in mitosis is not clear (see p. 159).

Figs. 27–29.—The chromatin is, in these stages, collected into the two poles which are connected by an achromatic spindle.

Fig. 30.—*Dendrosoma* feeding upon a *Euplotes* (*E.*). The swollen end of the arm of the *Dendrosoma* (*D.*) was $15\ \mu$ in diameter, the length of the *Euplotes* body $100\ \mu$. A stream of particles (*St.*) could be seen passing down into the arm through the attached suckers; the other suckers were quite indifferent.

Fig. 31.—Side view of a gemmula of *Lernæophrya* (?) showing two of the three contractile vacuoles. None of these gemmulae exhibited a micronucleus.

Fig. 32.—A very young, probably quite recently fixed specimen of *Lernæophrya* with only four suckers, showing a completely formed gemmula in position. There are no micronuclei to be seen.

Figs. 33 and 34.—Two sketches of young *Dendrosomas* showing the method of arm formation in *Dendrosoma*.

Figs. 35–39.—Series of studies of *Urnula epistylidis epizoic* on *Dendrosoma* showing the different forms assumed by the tentacles. In figs. 35–38 the specimens have only one tentacle, in fig. 39 it has two.

Fig. 40.—Section through an *Urnula* from a stained preparation. In this specimen the diameter of the body is $19\ \mu$, of the meganucleus (*M.*) $8.5\ \mu$, and of the micronucleus (*m.*) $1.7\ \mu$.

Fig. 41.—Transverse section through an *Urnula* after the formation by fission of a gemmula (*g*). Drawn to the same scale as fig. 40.

Fig. 42.—A specimen of *Urnula epistylidis*, showing the body retracted below the mouth of the test. Copied from Engelmann (5) (Pl. 30, fig. 13).

Fig. 43.—*Urnula epistylidis*, showing the formation of the gemmula. Copied from Claparède and Lachmann (5) (Pl. 10, fig. 3).

Fig. 44.—Free-swimming gemmula of *Urnula*. Copied from Claparède and Lachmann (5) (Pl. 10, fig. 3).

Fig. 45.—A portion of the body of an *Urnula* very much enlarged to show the spiral marking of the tentacle (*t.*).

Fig. 46.—A specimen of *Trichophrya epistylidis* (sp.?) found in the Bridgewater canal attached to the stalk of an *Epistylis*. (From a stained preparation.) No micronucleus could be seen. Size $129.5\ \mu \times 111\ \mu$.

Fig. 47.—A very young *Dendrosoma* with one arm and one micronucleus, also attached to an *Epistylis* stalk. The size of this specimen is $61.3\ \mu$ in greatest length, including the arm. From a stained preparation.

Fig. 48.—Another rather older *Dendrosoma* with three micronuclei. Size $60\ \mu$, + the arm $60\ \mu = 120\ \mu$. From a stained preparation.

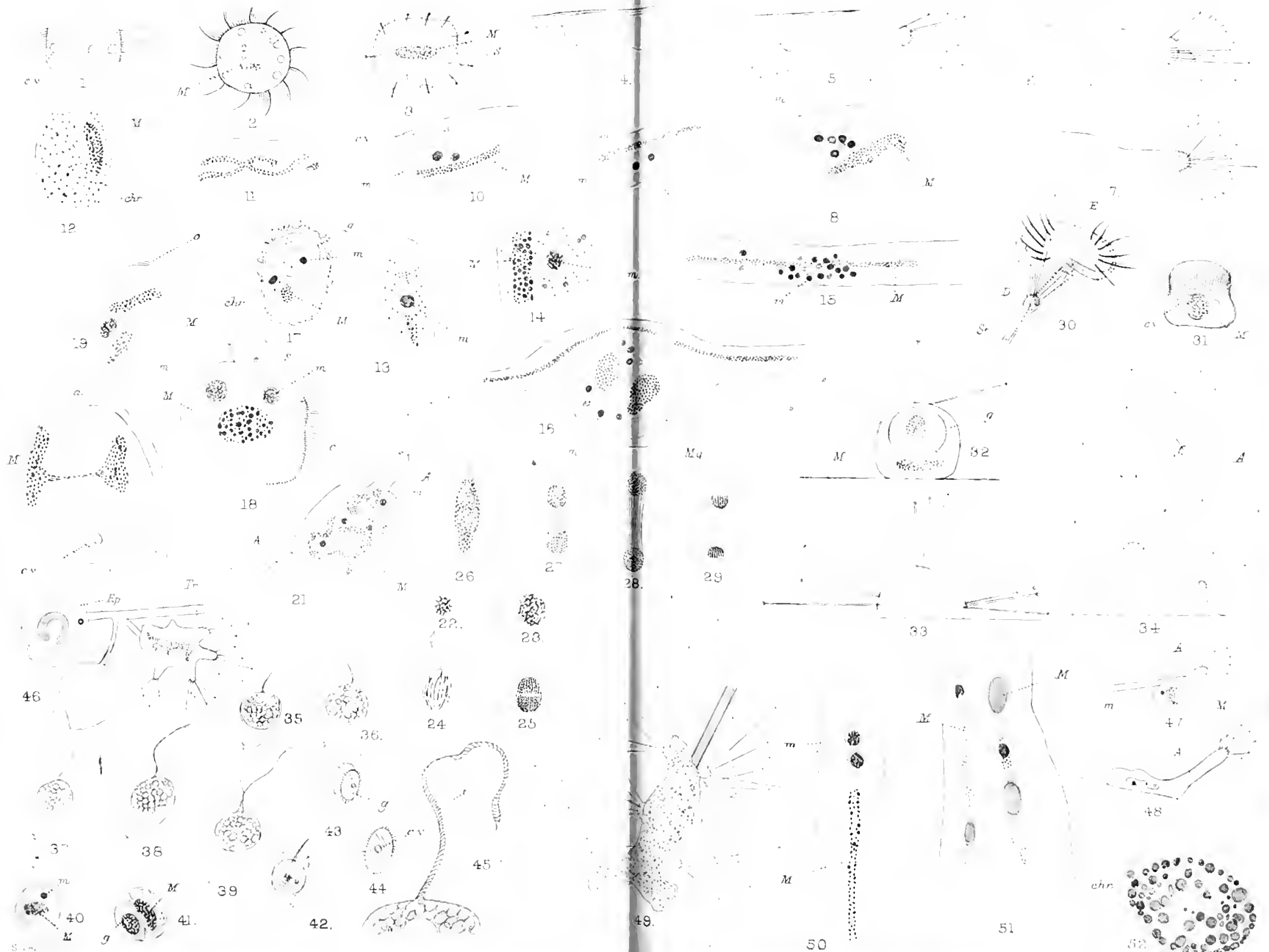
Fig. 49.—*Lernæophrya* (sp.?) from the Bridgewater canal. Drawn from a living specimen January, 1909.

Figs. 50–52.—Three figures drawn to the same scale ($\times 1000$) to show the varying structure of the meganucleus of *Dendrosoma*.

Fig. 50.—Section through a part of an arm (Birmingham material) in the region where the meganucleus terminates. The terminal extremity was in the direction of the upper side of the figure, but was not included in the actual section. Two micronuclei are seen beyond the meganucleus. The size of the largest granules was only $0.85\ \mu$.

Fig. 51.—Section through an arm showing two meganuclear bands. The chromatin granules are smaller than in fig. 50, but the meganucleus contains peculiar, large, irregular bodies which give the chromatin reaction.

Fig. 52.—Section through a meganucleus (Bridgewater canal material). The largest chromatin granules seen in this section are $2.5\ \mu$ in diameter.



On the Structure of the Excretory Organs of Amphioxus.

**Part 2.—The Nephridium in the Adult. Part 3.—Hatschek's
Nephridium. Part 4.—The Nephridium in the Larva.**

By

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With Plates 11—16, and 1 Text-figure.

Part 2.—The Nephridium in the Adult.

IN Part 1 of this contribution, which appeared some years ago (7), it was shown that the nephridia of *Amphioxus* bear a startling resemblance to the nephridia of certain Polychæte worms, such as *Phyllodice*; they are segmental, they are formed of an excretory canal opening to the exterior (atrium), but ending internally in blind branches; these blind ends are provided with typical solenocytes. The last fact was the only striking novelty then contributed to the descriptions of Weiss (13) and Boveri (1). Although I can now add but little of real importance to my previous account, it is necessary to return to the subject again owing to the publication by various authors of certain statements as to the presence of internal openings. These statements, if not soon disproved, will spread confusion and error in the literature of the subject which it may take years to eradicate. Had not other pressing work prevented me, I should myself long ago have attempted

to dispose of them. Such convincing evidence can now be brought forward against these views, that it may be hoped the question will soon be definitely settled. Moreover, I take this opportunity of adding certain details which serve to complete our knowledge of these interesting organs.

In 1904 Boveri published a note (1a) in which, while accepting my description of solenocytes, he still maintained that the lumen of the nephridial canal opens into the dorsal, or hyper-branchial, coelom by one or more funnels.

Felix, in his excellent account of the development of the excretory organs of the Vertebrata (2), fully adopted my view as to the structure of the nephridia of *Amphioxus*, and gave some figures, derived from Boveri's original paper (1), but "corrected after Goodrich." In these figures the funnels were closed up.

Shortly afterwards Felix changed his opinion, having examined Boveri's sections, and republished the latter's figures in their original condition (with open funnels) in a second work on the excretory organs of the Vertebrata (3). K. C. Schneider likewise accepts Boveri's description, but gives no new figures to support his opinion (12).

Now, it may at once be stated that I am firmly convinced that such internal funnels do not exist. Indeed, I am prepared not only to affirm that they do not occur in any *Amphioxus* I have examined, but also to prove the correctness of my description to any competent person who is willing to look at my preparations. My affirmation is based on a long and patient study of numberless specimens, both living and preserved. It is naturally to sections that one turns for the final verdict, and I may say that, although I have examined hundreds of sections of specimens of different sizes and ages, preserved according to a variety of methods, cut in all directions, stained in various ways, never once have I been able to discover such an opening. Occasionally, if the section is broken, the preservation defective, or the staining imperfect, one may meet with what at first sight appears to be a communication between the coelom and the nephridial

canal; but this deceptive appearance is soon exposed on a more critical examination of the preparation. Thick sections are especially misleading. No observation made on a section more than 5μ thick is in the least conclusive. The technical difficulties are very great in the study of *Amphioxus*; the tissues are brittle, the cells very small and difficult to stain satisfactorily. Formol and Flemming's fluid, corrosive-acetic, and picro-sulphuric-formol are all good preservatives. Great care must, however, be taken to avoid shrinkage, and for this purpose the method of double embedding in celloidin and paraffin is most useful. By far the best sections are obtained from pieces of the pharynx removed from the fresh animal, and preserved separately. One may use either carmine or hamatoxylin for staining the nuclei; but it is quite essential to add some suitable cytoplasmic stain such as acid fuchsin. For the particular purpose we are now concerned with, perhaps some strong staining reagent like Mann's methyl-blue eosin is the best for working out minute details under high powers, though picro-nigrosin also yields valuable results.

Turning now to the structure of the nephridium, we find the external pore opening at the very top of the atrial cavity, on the anterior outer surface of the secondary or tongue bar (*op.*, figs. 1, 2, 7, and text-figure). The pore leads into a canal which gives off a short posterior limb, and a much longer anterior limb. The latter passes forwards to the next primary bar, and downwards into the triangular coelomic cavity delimited by the ligamentum denticulatum. In a fully developed nephridium both the anterior and posterior limbs give off diverticula of varying length, which may sometimes branch. These are shown in fig. 1 of Part 1 (7), and are seen again in the reconstructions given in this paper (figs. 1, 2, 3).

Let us pass to the conclusive evidence which can only be obtained from sections. The wall of the nephridial canal contains many nuclei (figs. 7, 13). In some places they are so closely packed that they seem to press against each other.

In other regions of the canal they may be more sparsely distributed. Cell outlines are rarely visible. The cytoplasm

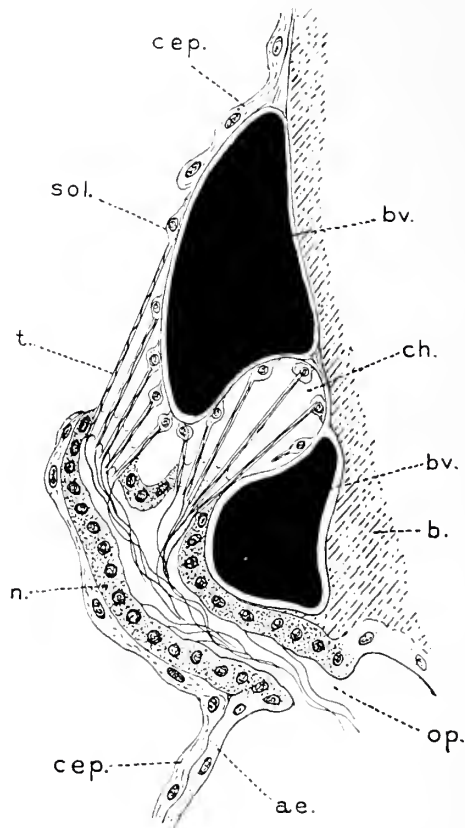


Diagram of a section through the nephridium, passing along a plane at right angles to the long axis of the animal, and parallel to the gill bar.

a. e. Atrial epithelium. *b.* Base of secondary gill-bar. *bv.* Blood-vessel. *c. ep.* Cælotomic epithelium. *ch.* Chamber containing solenocytes. *n.* Wall of nephridial canal. *op.* Nephridiopore. *sol.* Solenocyte cell, and *t.* its tube containing a flagellum.

usually contains numerous granules of an excretory nature. As we approach the tip of a diverticulum, we find that the

nuclei do not gradually decrease in number, but suddenly stop in the immediate neighbourhood of the solenocyte tubes (figs. 6, 20). Here, where these tubes spring out of the canal, there are no nuclei; but the wall itself is continued as a sheet of more or less granular cytoplasm completely closing off the lumen of the canal (figs. 6, 9, 20, 21). This canal wall may be thick or thin, the variation in thickness depending, I believe, chiefly on the state of tension of the fluid inside the canal. In good thin sections the wall is always visible. Indeed, the better the section, and the more perfect the stain, the clearer becomes the limiting wall, whatever may be the direction in which it is cut.

Figs. 19 and 20 represent two sections taken parallel to the surface of the nephridium, sagittal sections of the animal. The first just shaves through the outer wall of the canal, and shows many solenocytes lying on the blood-vessel. The second, which only corresponds to the left hand portion of the first figure, cuts deeper into the canal through the extremity of one of the branches, where may be seen the solenocyte tubes piercing the closing wall. In the next section the nuclei of the opposite side begin to appear, the whole thickness of the small solenocyte-bearing offshoot having been nearly cut through. The following section would show only a slice of the wall. There is no opening. Fig. 21 gives a similar view of another nephridium in the same animal.

Two consecutive sections through the lowermost tip of the anterior limb of the nephridium are drawn in figs. 13, 14. Here again are seen the tubes piercing the wall, in which there is no trace of an opening.

Figs. 5 and 6 represent sections from a series nearly transverse to the animal and parallel to the bar. That in fig. 5 passes through the external pore, and shaves off the wall of a diverticulum. The next section (fig. 6) cuts through the extremity of this diverticulum. It is seen that the lumen is closed off from the cœlom by a distinct cytoplasmic wall, through which pass solenocyte tubes. In fig. 7 is drawn a portion of the same section when the microscope has been

focussed to the lower surface; the nuclei of the wall are again visible. There is no opening.

Innumerable figures could be given of series of sections all telling the same story. But the critic will say: if the diverticula are really closed, sections taken at right angles through their tip should show the tubes cut across embedded in the thickness of the wall. Such sections are not difficult to find, and I figure several on Plates 12 and 13.

Figs. 10 and 12 represent two consecutive sections across the tip of a branch. In the first are seen the tubes entering the wall, while the next (fig. 12) strikes the lumen. A small part of this figure is shown slightly diagrammatised (fig. 11) on a larger scale. Again three consecutive sections are drawn in figs. 15, 16, and 17. Here two sections cut through the solid wall before the lumen is reached. Lastly, fig. 18 represents a section through two adjacent processes, one of which has been cut so as to expose the lumen, while the other shows very clearly the solenocyte tubes piercing the wall and embedded in its cytoplasm.

The evidence of all these sections is quite unequivocal; it would serve no good purpose to multiply instances; there is no opening, the wall is continuous, and is traversed by the tubes of the solenocytes.

But there is other evidence of a different nature leading to the same conclusion. I have observed in a living nephridium the fluid inside the nephridial canal so compressed, perhaps by the overlying cover-glass, that it dilated the tip of the diverticulum so as to give rise to a bulging vesicle at its extremity. Now, such a swelling could obviously not be formed if the tip were open.

We may now turn to injections to corroborate our view. I have recently injected the dorsal hyperbranchial cœlom with Indian ink. The minute black particles were held in suspension in sea-water. Such a fluid, if introduced with a hypodermic syringe, can be made to fill the cœlom. It is clear that if the nephridium communicated with the cœlom the ink would penetrate into the canal; this would happen

all the more easily, since a powerful ciliary current works towards the external pore. Sections of such injected specimens show conclusively that not a single particle of ink has entered the nephridial canal, although the ink has penetrated into every chink of the cœlom.

But, it may be asked, if the facts are so plain and conclusive, how is it that so keen-sighted and accurate an observer as Boveri has been deceived? Well, if it will not be considered presumptuous on my part, I will attempt to explain how the mistake arose.¹ To begin with, the sections he examined were not appropriately stained. The nuclei are clear, but the cytoplasm scarcely stained at all. In the majority of the sections which I had the opportunity of seeing the wall which closes the tips of the diverticula was very difficult to make out, though I could detect it on close examination in a suitable light. I naturally turned with great interest to the section given on Pl. 33, fig. 17, of the original memoir (1), and of which a photograph is published in the 'Anatomischen Anzeiger' (1a). Anyone on first looking at this section might be led to believe in the existence of a funnel. The appearance is extraordinarily deceptive. But it is deceptive, and the deception is due to two things. First of all the nuclei are deeply stained, but the cytoplasm practically colourless and transparent; in the second place the section is thick. The figure given by Boveri is really an optical section of the preparation. The closing wall can, indeed, be seen, but only with the greatest difficulty. The misleading appearance of a funnel is due to the sudden cessation of the nuclei round the base of the solenocyte tubes; an appearance which is further heightened by the limit of the cœlomic epithelium at the same spot (see p. 193, and text-figure).

Let it not be thought that in insisting on the absence of an opening I am unduly influenced by *à priori* considerations

¹ Soon after the publication of his paper (1a) I wrote to Professor Boveri, who then very kindly sent me his preparations, and I gladly take this opportunity of thanking him for his courtesy.

due to theoretical bias. It is true that I hold that the renal organ of *Amphioxus* is a nephridium homologous with the nephridia of Annelids and Platyhelminths, and not homologous with the kidney tubules of the Craniata (5, 7); but it is now well known that the true nephridia of Annelids may open into the cœlom. There is no *à priori* reason why they should not do so in *Amphioxus*. However, no nephridium has yet been found possessing both solenocytes and an internal opening, though such intermediate stages must presumably have existed.

The Relation of the Nephridium to the Blood-supply.—The general blood-supply has been well described and figured by Boveri (1). But according to my observations the vessels occur not so much as narrow capillaries, as in the form of a large expanded vessel spreading over the area occupied by the excretory organ. This is shown in sections (figs. 7, 23), and also in the reconstructions given on Plate 11. It will, moreover, be noticed that, although the greater part of the bloodvessel lies on the inner or atrial surface of the nephridium, yet several loops pass round to the outer or cœlomic surface. Thus a considerable part of the nephridial canal is entirely surrounded by the bloodvessels. The solenocytes radiate out from the canal, and always lie on the wall of a bloodvessel, being attached to it by a protoplasmic process (figs. 4, 15). The way in which these cells are distributed is shown in figs. 14, 19, and diagrams 2 and 3, and the text-figure. It will there be seen that the longer tubes, which are of course those belonging to cells furthest away from the canal, pass over the shorter tubes to reach their destination. Never do the solenocytes project freely into the cœlom; when they appear to do so in sections this is, I believe, due to the cell having become detached accidentally, either during the process of preservation or of cutting. The tubes are therefore fixed at both ends.

In the text-figure may also be seen the peculiar disposition of the solenocytes at the top of the secondary gill-bar. Here

the canal of the nephridium gives off two or three short diverticula, which are turned away from the cœlom towards the middle line. The numerous solenocytes projecting from these diverticula lie in a sort of pocket or chamber (figs. 1, 2, 3, 23), which only communicates with the cœlom by means of a dorsal opening, over which pass a large number of solenocyte tubes. In one region the inner wall of this chamber is formed by the skeletal rod of the gill-bar (figs. 3, 23). Somewhat similar pockets are found occasionally in connection with other parts of the nephridium, as, for instance, the anterior limb of the canal. The cavity in the chambers is, I believe, rather of the nature of a lymph space than of a true cœlomic cavity.

The Relation of the Nephridium to the Cœlomic Epithelium.—It is important to determine exactly what is the disposition of the cœlomic epithelium in the neighbourhood of the nephridium. Boveri (1) and Weiss (13) have already shown that the canal is covered by the cœlomic epithelium; but this epithelium only clothes the outer or cœlomic surface (text-figure). It passes on to the nephridium from the atrial wall, covering the canal and its blind branches to their extremity. Here it is not reflected so as to pass over to the inner or atrial surface of the organ, but ends abruptly near the base of the solenocyte tubes (figs. 2, 6, 8, 9).

Thus the nephridium and the bloodvessels which accompany it may be said to lie "morphologically" entirely outside the cœlom; between the cœlomic epithelium and the atrial epithelium. The nephridium is, in fact, retroperitoneal. This is true, I believe, of the solenocytes themselves, though less easy to prove. For the cœlomic epithelium stops short where the solenocytes begin (figs. 6, 8, 2), passing neither on the inner side over the bloodvessel, nor outside them over their cœlomic surface. For a long time I was under the impression that a very delicate membranous extension of the epithelium covered over the cœlomic surface of the solenocytes; but I am now satisfied that this is not the case,

although sometimes the epithelium seems to stretch over the base of the solenocyte tubes for a considerable way. The cœlomic epithelium is not continuous with the wall of the canal at the tip of the diverticula, but often can be seen in sections to end with a free and jagged edge. Over the region where the solenocytes occur there is a gap in the cœlomic epithelium, so that cœlomic fluid freely bathes the solenocyte tubes (figs. 2, 3). That the space in which lie these tubes, and even the deep pockets described above (p. 193), communicate with the cœlom is evident in specimens injected with Indian ink.

Since no epithelium covers the solenocytes their true relation to the cœlom cannot be made out for certain in the adult. Without going into the question of their development in this paper, I may say that a careful examination of M. Legros's excellent preparations has convinced me that in the very earliest stages of its development the whole rudiment of the nephridium and solenocytes lies enclosed between the cœlomic epithelium and the atrial wall. There is nothing unusual in the solenocytes coming into secondary contact with the cœlomic fluid. We know that in the *Actinotrocha* larva the nephridium pierces the wall of the preseptal hæmocœl, and the solenocytes project freely in the blood (8). In many Polychætes also the nephridium passes through the cœlomic epithelium and the solenocytes lie naked in the cœlomic fluid (6).

To sum up the chief points in this contribution:—The careful examination of the nephridia in sections and in the living state shows that they have no internal opening. The tubes of the solenocytes pierce the wall of the nephridial canal, and open into its lumen. The flagellum passes down the tube into the lumen. The solenocytes are attached to the wall of the bloodvessels, which expand in this region, and may surround the canal. Both the bloodvessels and the nephridial canal are covered by the cœlomic epithelium, being situated between it and the atrial wall. Over that region which is occupied by the solenocytes there is a gap in

the cœlomic epithelium, allowing the fluid to bathe the tubes. Nevertheless the whole excretory organ is to be considered as retroperitoneal.

Part 3.—The Nephridium of Hatschek.

This organ was first described by Hatschek in a paper without illustrations (9), wherein he states that the first somite of the left side divides into two halves, of which the first acquires an opening to the exterior, and becomes the "Räderorgan" or ciliated glandular pit in the buccal cavity, while the second and inner half becomes the nephridium. "Es entwickelt sich in der Larve als mesodermaler wimpernder Trichter und canal, und zwar nur linkerseits vor der Mundöffnung, in der Region des ersten Metamers; es wächst später weiter nach hinten aus. Bei dem ausgebildeten Thiere erstreckt sich das Organ an der linken Körperseite längs des ventralen Randes der Chorda von nahe dem vorderen Mundrande bis dicht hinter das Velum. Hier scheint es in den Kiemendarm zu münden (die Ausmündung muss ich nochmals prüfen). Es liegt in einem engen Fortsatz der Leibeshöhle überlagert von der linken Carotis" (9).

Lankester and Willey incidentally refer to the organ in their important memoir on the larva of *Amphioxus* (10), and there state that "in the condition in which we have observed this structure (*viz.* in larvæ ranging from the stage with three gill-slits up to closure of the atrial cavity) there does not seem to be any special reason for regarding it as a nephridium. We should prefer to call it the subchordal tube. It appears to end blindly anteriorly, and to open into the buccal cavity near the recurved extremity of the glandular tract which accompanies the club-shaped gland."

Willey in his later contribution (15) incompletely represents the nephridium of Hatschek in his figures, but actually draws the solenocytes without, however, realising their significance. He says, "I could not certainly detect cilia in it, and, in fact, was unable to understand its import. It seems to possess a superficial resemblance to the head-kidney

of Annelid larvæ (trochosphores), but I can form no opinion as to the reality of any such resemblance."

The next author to mention the organ is MacBride, who briefly describes its development, believing that it arises from the communication between the gut and the second myotome (11).¹

Van Wijhe (14) describes the canal of Hatschek's nephridium in the adult, applying to it the name Schlundforsatz: "eine enge Röhre, welche dem linken Seitenrande der linken Aorta angeschmigt ist. Das enge lumen wird von einem einschichtigen Cylinderepithel begrenzt und bildet streckenweise seitliche Ausbuchtungen. Wo eine solche angeschnitten wird, können zwei Lumina im Schnittbilde auftreten. Unmittelbar hinter dem Velum mündet die Röhre mit einer feinen Öffnung in den Schlund aus." He denies, however, the presence of the cœlomic cavity described by Hatschek, and does not accept the latter's theory as to the organ's function. "Nach meiner Meinung," says van Wijhe, "ist das organ nicht anderes als ein Rudiment des vorderen Darmendes, welches beim Embryo in das Flimmersäckchen (linke Entodermsäckchen) ausmündete."

It is to Goldschmidt that we are indebted for the first description of solenocytes in the nephridium of Hatschek (4), placing its homology with the posterior nephridia beyond doubt. His account seems, however, to be based on imperfect material, and he falls into the error of ascribing to the canal an internal opening such as Boveri had described in the paired nephridia.

I have recently had the opportunity of studying this interesting organ in adult and larval specimens in Helgoland,² and am thus able to give a more complete description of it.

¹ I am unable to agree with the view of either Hatschek or MacBride as to the origin of this nephridium.

² I gladly seize this opportunity of thanking Prof. Heineke, Prof. Hartlaub, and the staff of the Königl. Biologische Anstalt for the kind way in which they received me in Helgoland.

The nephridium of Hatschek reaches its maximum development in the adult, where it is indeed the largest nephridium in the body, some 2 mm. in length. Lying on the left side, below and parallel to the notochord, it opens just behind the velum into the pharynx,¹ and runs forward a long distance to a point just in front of the ciliated groove (Räderorgan). Here it ends blindly, and along its course are given off short blind diverticula (figs. 27, 42, 43, 44). Solenocytes are set on the dorsal and lateral surfaces of the organ along almost its whole length, being especially numerous on the diverticula (fig. 28). Altogether an enormous number of solenocytes are present on this nephridium in the adult *Amphioxus*.

The canal runs along the floor of a narrow cavity beside the aorta (figs. 42—44). It is to the wall of this cavity that the solenocytes are attached, and it appears to be of coelomic nature; at all events it is in open communication with the myocœle of the first myotome in larval stages (figs. 25, 26, 33). In the adult, however, it is closed off, and the lining epithelium seems to be very irregularly developed, forming no distinct layer of cells (fig. 28).

In the larva of about 13 gill-slits, of the left series only (fig. 33), the nephridium can be well seen by transparency as a short tube opening behind into the pharynx (fig. 24). Its dorsal surface is entirely beset with solenocytes in several closely packed rows (fig. 29). An optical section of the organ at this stage is represented in fig. 38, showing clearly the way in which the tubes of the solenocytes pierce the thin dorsal wall.

We may summarise as follows the observations recorded above:—The nephridium of Hatschek is a true nephridium, similar in structure to the posterior paired nephridia. In the adult, where it reaches its maximum development, it extends along the left aorta from in front of the ciliated

¹ On one occasion only I have found an opening from the canal into the hinder region of the buccal cavity itself, as well as the posterior opening into the pharynx.

groove backwards to the pharynx into which it opens. Very numerous solenocytes are set chiefly on short blind diverticula. It has no internal opening, and lies in a cavity, which is in communication with the myocœle of the first myotome in the larva.

That this nephridium is in every way similar to and homologous with the paired posterior nephridia there can be no doubt. Van Wijhe's suggestion, mentioned above, must therefore be abandoned. Two peculiarities, however, still remain to be explained; its unpaired character and its opening into the alimentary canal. No one, so far as I am aware, has yet worked out the exact relation of the gill-slits to the somites in the larva of *Amphioxus*, and my own observations on this point are very incomplete. But judging from the course of the dorsal spinal nerves (fig. 30), the first gill-slit of the left (on the right side) series, which is the first to appear in the larva, corresponds to the third myotome. Probably its true morphological position is between the second and third myotome. Presumably Hatschek's nephridium would correspond to the next gill-slit in front, between the second and first myotomes, did such a slit exist. As for its unpaired character, I can for the present offer no better explanation than this, that it is the left of an original anterior pair of nephridia, the one-sided development of which is no doubt correlated with the general asymmetry of the anterior region so conspicuous in the larva. But this question can only be profitably discussed after an exhaustive study of the development, and must therefore be put aside for the present. In the same way a detailed knowledge of the development of this organ, and of the posterior nephridia, is necessary before one can discuss the significance of the anomalous position of the opening.

Part 4.—The Development of the Left Series of Nephridia in the Larva.

For many years I have been trying to trace the development of the nephridia in *Amphioxus*. In 1902 I collected a large amount of material from the Pantano at Faro; but ill-health prevented my working out the development on the living larva, and I failed to do so on the preserved specimens. It was not till last year that I was again able, in Helgoland, to study the living larva, and succeeded in tracing some stages of the development of the excretory organs. In the meantime Legros had been studying the same subject in Naples, and published anonymously a preliminary notice of his results a short time ago (16).¹

In the present paper I shall not discuss in detail the first origin of the nephridia, but restrict myself to a description of the stages found in the larva with from ten to fifteen gill-slits of the left hand series, and no trace of the right hand series. These are the only stages which I have been able to study sufficiently in the living state.

Fig. 30 gives a left side view of a young larva with eleven slits. The anterior gill-slits are still well on the right side, but the hinder slits are in or near the middle line. The future dorsal edge of each slit may, of course, at this stage be more ventral than the future ventral edge. The nephridia are seen as small rounded sacs near the posterior ventral corner of each slit. Every slit from the first to the last has such a nephridium. At this early stage there is no atrium, the slits have an internal margin of thick branchial epithelium, which is thrown into characteristic folds when the branchial muscles contract, while the external margin of the slit is formed by a thin fold of the body wall, acting as a sort

¹ Through the kindness of M. Legros I have had the opportunity of examining his sections, and I cannot agree with his conclusions as to the origin of the nephridia from the coelomic epithelium, nor as to the presence of internal openings. But I believe he has modified his views considerably on these points since the publication of the note.

of sphincter (figs. 33 and 36). A shallow branchial chamber lined with epidermis is thus formed, leading from the external to the internal opening. It is in this chamber that the nephridium opens, at a place corresponding apparently to the point of junction of the ectoderm with the endoderm (fig. 41). The position of the nephridiopore can be seen in figs. 36 and 39. When the atrium becomes formed by the closing off of the space between the metapleural folds, with which the branchial cavities become merged, the pores open into the atrium. A ventral view of a stage where the atrium has just begun to be formed posteriorly shows one or two nephridia behind the last open gill-slit (fig. 35). Probably these nephridia belong to the posterior gill-slits, which have closed up (Wiley, 15); they open now directly on the surface (fig. 30).

The young nephridium is a flattened sac, without internal opening (figs. 36 and 39). From its inner end spring a large number of solenocytes; their tubes pierce its wall, and their flagella pass into the lumen of the sac. The majority of the solenocytes spread over the blood-vessel which runs along the future dorsal edge of the slits. The solenocytes of the first few slits scarcely extend beyond this limit; but, passing backwards to more posterior nephridia, we find that the solenocytes spread farther and farther up towards the dorsal aorta, the tubes lengthening out as the cells lie farther from the nephridial sac. At about the fifth or sixth nephridium some of the solenocytes actually reach the aorta (fig. 40). The tubes in this case may attain a really astonishing length, stretching right across the field of a $\frac{1}{12}$ th oil-immersion objective with oc. 8.

Fig. 34 represents the posterior gill region of a living larva, in which the remarkable development of the solenocytes is well shown. Here a group of the longest solenocytes, some twelve to eighteen in number, spread out over the aorta in a most beautifully regular fan-like arrangement in each segment. A section of this region is shown in fig. 31; the fan-like disposition is found in each segment to the hindmost

limit of the series of nephridia. Presumably the dorsal solenocytes degenerate later, since they are not known to exist in the adult.

The observations on the larval nephridia recorded in this part may be summarised as follows:—To every gill-slit corresponds a nephridium, consisting of a sac closed internally, but opening to the exterior apparently at the point where the ectoderm joins the endoderm in the shallow branchial chamber. From the internal blind end of the nephridial sac spring numerous solenocytes, some of which reach and spread over the aorta at every segment in a fan-like arrangement. This structure is only fully developed from about the eighth segment backwards to the last nephridium.

July 3rd, 1909.

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LIST OF REFERENCE LETTERS.

a. e. and *a. ep.* Atrial epithelium. *ao.* Aorta. *b.* Secondary gill-bar. *b. c.* Buccal cavity. *b. ep.* Epithelium of buccal cavity. *br.* Brain. *br. c.* Branchial epithelium. *bv.* Blood-vessel. *ca.* Cavity in which runs Hatschek's nephridium. *c. ep.* Cœlomic epithelium. *c. gl.* Club-shaped gland. *ch.* Solenocyte chamber. *cr.* Cirrus. *c. w.* Cut wall of nephridial canal. *d. n.* Dorsal nerve. *e. g. s.* Edge of gill-slit. *e. m.* Margin of mouth. *end.* Endostyle. *e. op.* External opening of club-shaped gland. *ep.* Epidermis. *fl.* Flagellum. *g.* Glandular area. *g. m.* Branchial muscle. *g. s.* ¹⁻¹⁵. Gill-slits. *gt.* Wall of gut. *h. c.* Pre-oral head-cavity. *H. neph.* Hatschek's nephridium. *ibw.* Inner blood-vessel. *i. op.* Internal opening of club-shaped gland. *l.* Lumen of nephridial canal. *l. p.* Lower margin of mouth. *mel.* Myocœle. *mt.* ¹⁻². First and second myotome. *mt. f.* Metapleural fold. *my.* Myotome. *n.* Wall of nephridial canal. *neph.* Nephridium. *n. c.* Nerve cord. *nc.* Nerve from left side. *nt.* Notochord. *op.* External opening of nephridium. *pb.* Peripharyngeal ciliated tract. *p. m.* Pre-oral muscle. *p. o. m.* Anterior oral muscle. *p. t. m.* Posterior oral muscle. *sol.* Solenocyte. *s. s.* Pre-oral sense-organ. *t.* and *tb.* Tube of solenocyte. *up.* Upper margin of mouth. *v.* Velum. *v. b. v.* Ventral blood-vessel now on right side. *vt.* Velar tentacle.

EXPLANATION OF PLATES 11—16,

Illustrating Mr. Edwin S. Goodrich's paper "On the Structure of the Excretory Organs of Amphioxus."

PLATE 11.

FIG. 1.—Diagrammatic reconstruction of a left nephridium and the neighbouring blood-vessels, from a series of sections taken parallel to the gill bar. The solenocytes are not represented. Side view from the outside. *Op.* indicates the position of the nephridiopore on the opposite side.

FIG. 2.—Similar reconstruction of a right nephridium, from a series of sections transverse to the gill-bar. In two places the cœlomic epithelium and the solenocytes are shown.

FIG. 3.—Reconstruction of a portion of a left nephridium, the blood-vessels, and the top of the secondary gill-bar, seen from behind.

FIG. 4.—Small portion of a section shaving off the wall of a nephridial canal, and showing the bases of the solenocyte tubes embedded in the cytoplasm. Cam. Z. 2 mm. ap. oil-imm., oc. 8.

PLATE 12.

FIGS. 5 and 6.—Two consecutive sections, parallel to the gill-bar, through a nephridium, showing the solenocyte tubes passing through the thickness of the wall of the canal. Cam. Z. 2 mm. oc. 4.

FIG. 7.—Drawing of the lower surface of the section of which the upper surface is represented in fig. 6. Cam. Z. 2 mm. ap. oil-imm., oc. 4.

FIG. 8.—Section across the anterior limb of a nephridium, showing the cœlomic epithelium passing over the outer surface of the canal.

FIG. 9.—Similar section showing solenocyte tubes piercing the wall of the canal. Cam. Z. 2 mm. ap. oil-imm., oc. 4.

FIG. 10.—Section parallel to a gill-bar, cutting the wall of a diverticulum of the nephridial canal (at *tb.*). Cam. Z. 2 mm. ap. oil-imm., oc. 4.

FIG. 11.—Diagrammatic view of a small portion of the wall of the diverticulum in the same section, showing the bases of the solenocyte tubes.

FIG. 12.—Next section to that drawn in fig. 10.

FIGS. 13 and 14.—Two consecutive sections through the ventral end of the anterior limb of a nephridial canal. Cam. L. $\frac{1}{2}$ oil-imm., oc. 3.

PLATE 13.

FIGS. 15, 16, and 17.—Three consecutive sections, parallel to a gill-bar, through the extremity of a diverticulum of the nephridial canal. Cam. Z. 2 mm. ap. oil-imm., oc. 12. In figs. 15 and 16 the solenocyte tubes are cut in the thickness of the wall of the canal.

FIG. 18.—Section across the ends of two adjacent nephridial diverticula. The bases of solenocyte tubes are clearly seen embedded in the cytoplasmic wall. Cam. Z. 2 mm. ap. oil-imm., oc. 18.

FIG. 19.—Longitudinal section cutting the surface of a nephridium. Cam. L. $\frac{1}{2}$ oil-imm., oc. 3.

FIG. 20.—View of the portion of the next section corresponding to the left-hand region of fig. 19.

FIG. 21.—Similar section of another nephridium.

FIG. 22.—Diagram to illustrate the direction of the sections drawn in figs. 5, 9, 10, 13, 15, and 19.

PLATE 14.

FIG. 23.—Section across the top of one primary and two secondary gill-bars, showing the position of the solenocyte chambers (*ch.*), and of the blood-vessels. The position of the external pore at a lower level is indicated by a cross X.

FIG. 24.—Transverse section of a larva, passing through the mouth, and opening of Hatschek's nephridium. Cam. Z. D., oc. 3.

FIG. 25.—Transverse section farther forward passing just beyond the anterior end of Hatschek's nephridium, where the cavity in which it lies opens into the first myocoel.

FIG. 26.—More enlarged view of a portion of the next section, showing the solenocyte tubes in a cavity continuous with the first myocoel.

FIG. 27.—Anterior end of an adult *Amphioxus*, ventral view. The buccal cavity has been opened up by cutting along the mid-ventral line. Hatschek's nephridium is seen on the left side of the notochord.

FIG. 28.—Small portion of a transverse section of the head, showing Hatschek's nephridium. Cam. L. oil-imm., oc. 3.

FIG. 29.—Similar view of a larva (the same as that in fig. 24, from Helgoland, with about thirteen gill-slits). Cam. L. $\frac{1}{12}$ oil-imm., oc. 3.

FIG. 30.—Portion of a longitudinal section of a larva, showing a nephridium opening behind the last open gill-slit. Cam. Z. 2 mm. ap. oil-imm., oc. 4.

FIG. 31.—Portion of a longitudinal section of a larva, showing the fan-like group of solenocytes on the aorta. Cam. Z. 2 mm. ap. oil-imm., oc. 4.

PLATE 15.

FIG. 32.—Left side view of a larva, drawn from living and preserved specimens.

FIG. 33.—Left side view of the anterior region of a slightly older larva on a larger scale, from living and preserved specimens. The cilia are not indicated.

FIG. 34.—Left side view of the posterior branchial region of a larva, showing the disposition of the solenocytes. From the living.

FIG. 35.—Ventral view of a region of a larva, showing the last open gill-slit, and two more posterior nephridia. From the living.

FIG. 36.—Ventral view of two posterior gill-slits of a living larva.

FIG. 37.—Solenocytes from Hatschek's nephridium in the larva.

FIG. 38.—Optical section of Hatschek's nephridium in the larva. From the living.

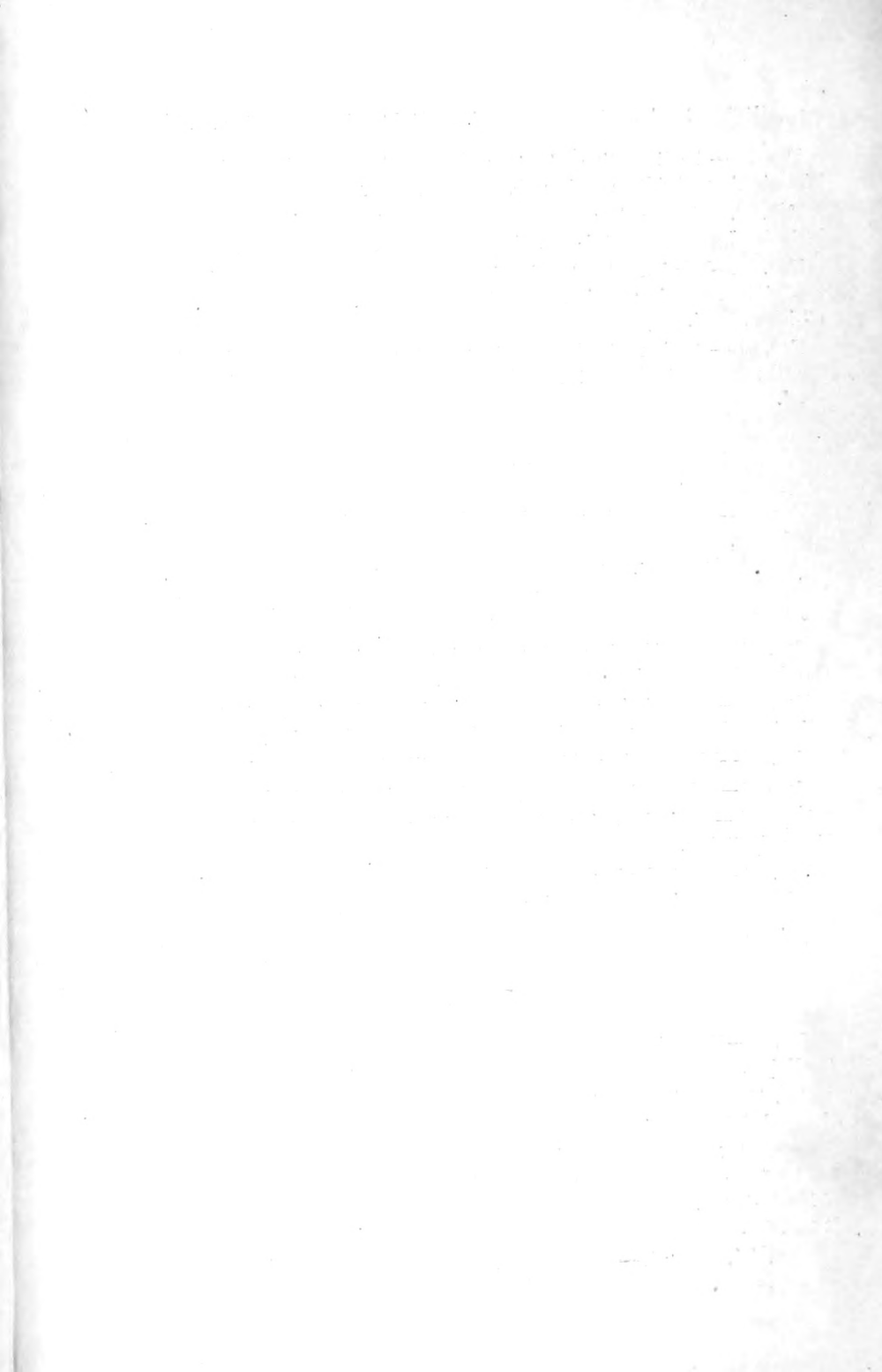
FIG. 39.—Ventral view of a nephridium showing its opening just within the margin of a posterior gill-slit in a larva. Solenocytes cut short.

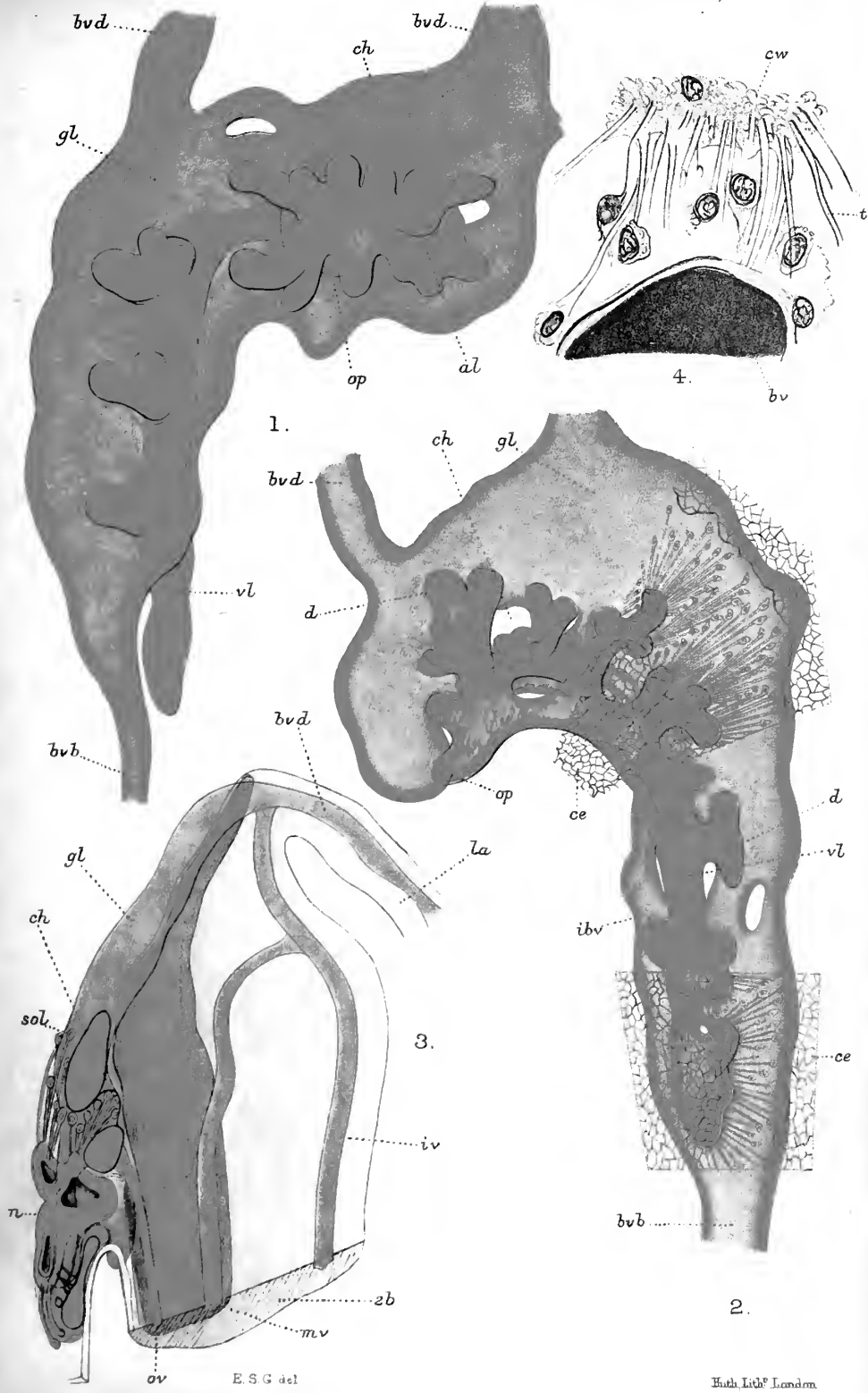
PLATE 16.

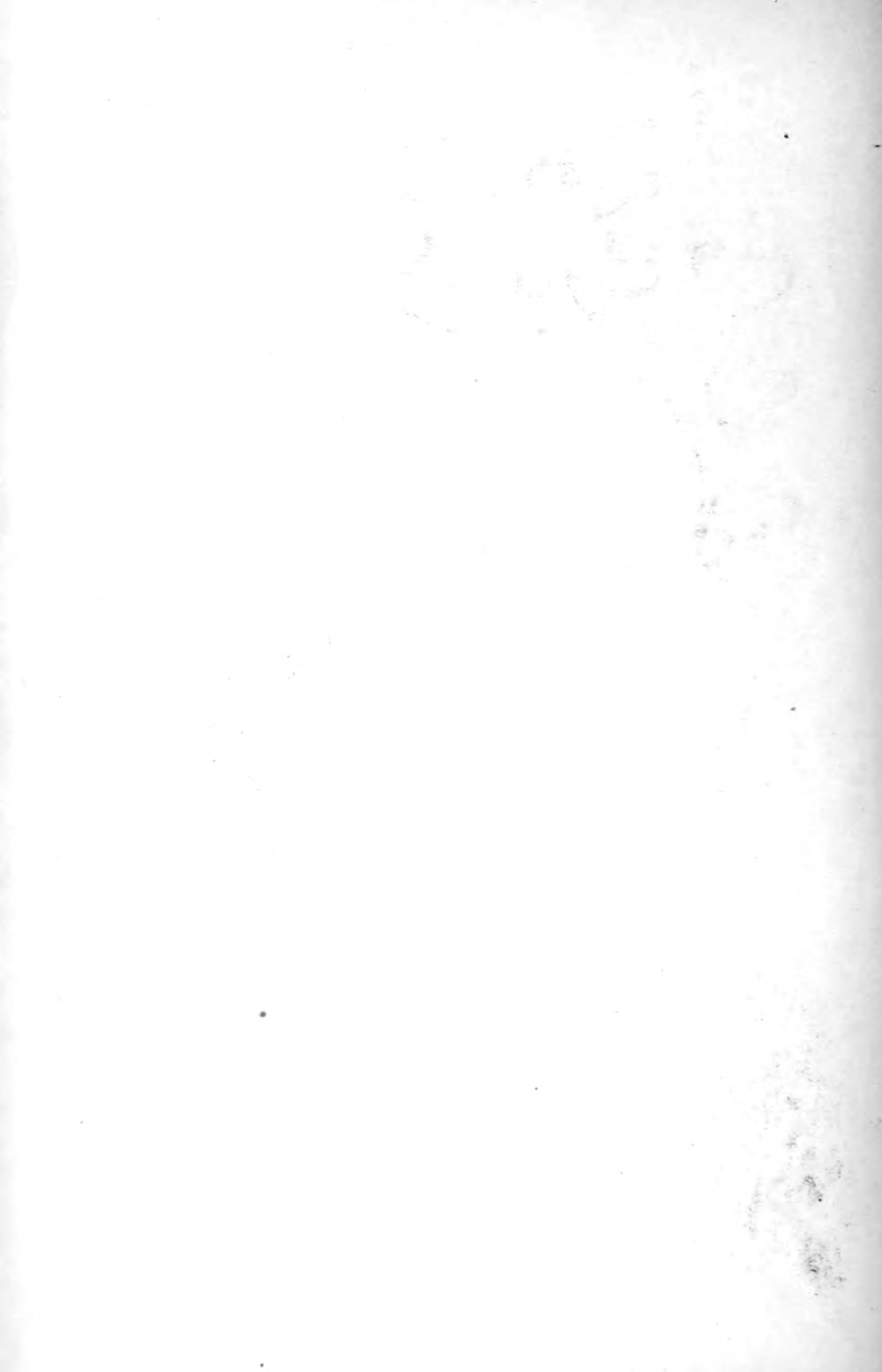
FIG. 40.—Left side view of a single nephridium in a larva. From the living.

FIG. 41.—Portion of a transverse section of a larva, passing through the nephridiopore. Cam. Z. 2 mm. ap. oil-imm., oc. 4.

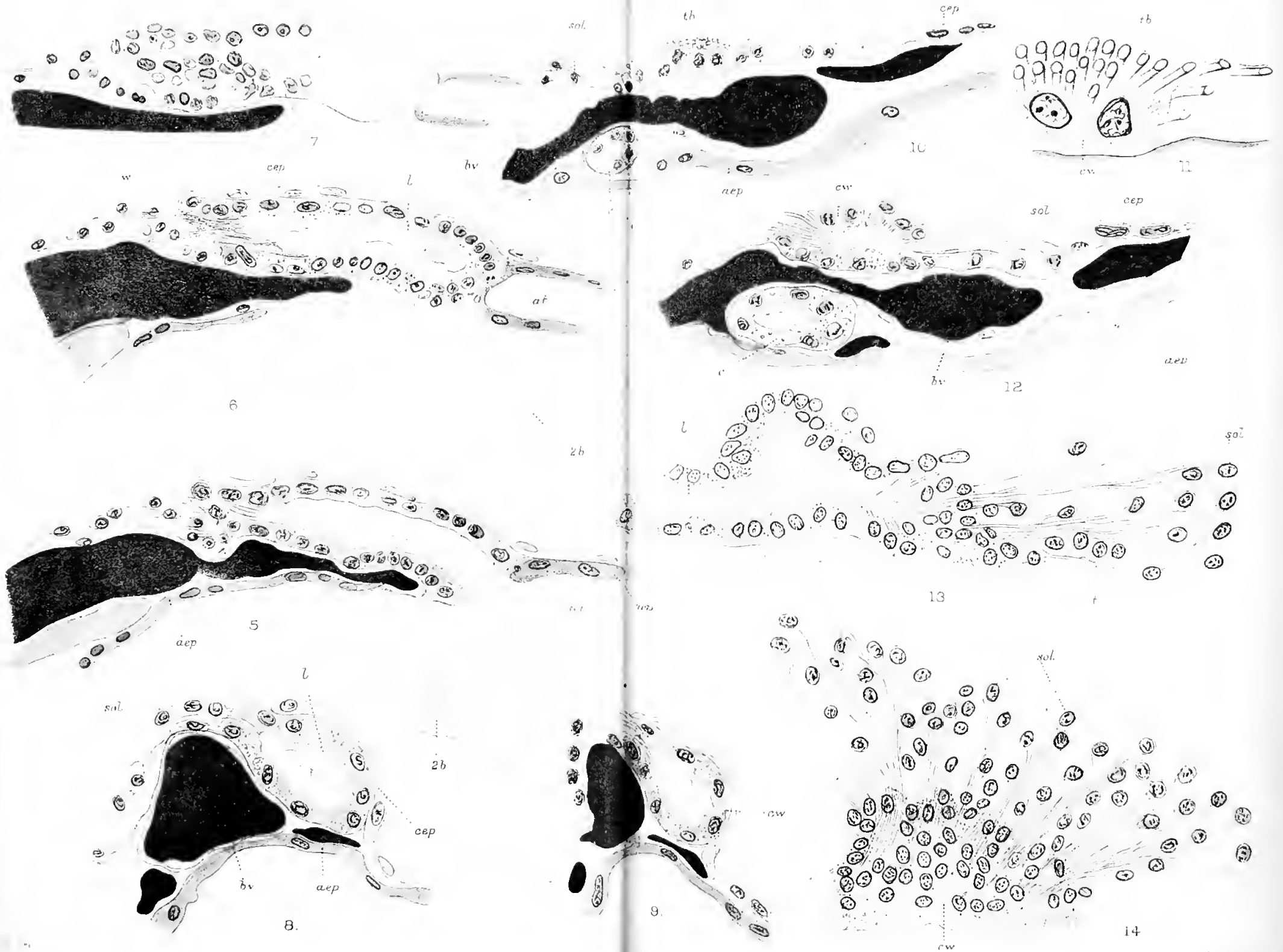
FIGS. 42, 43, and 44.—Portions of three transverse sections of the head of the adult, showing Hatschek's nephridium. In front of the ciliated pit (fig. 42), at the level of the ciliated pit (fig. 43), and behind it (fig. 44).





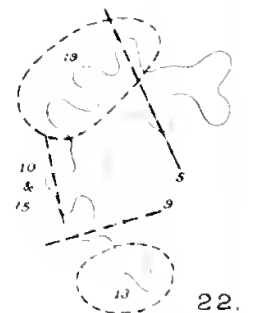
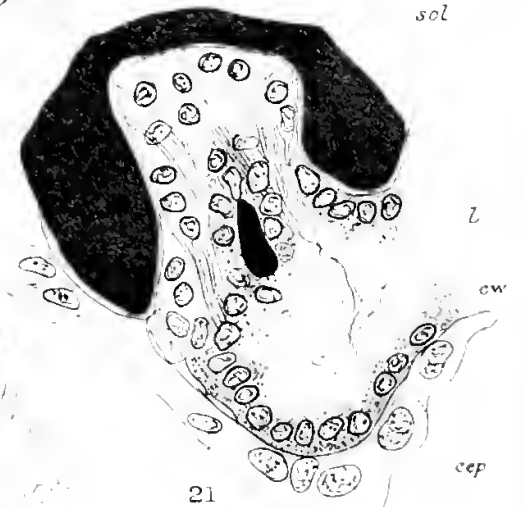
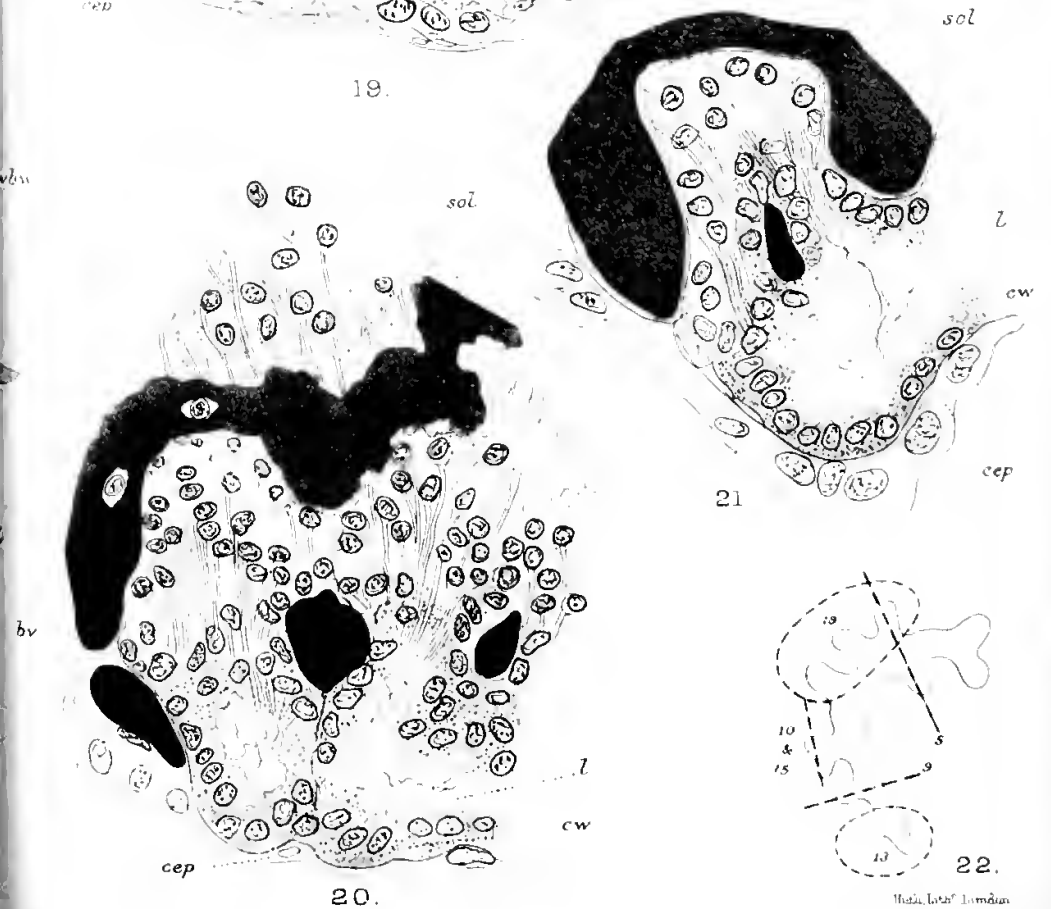
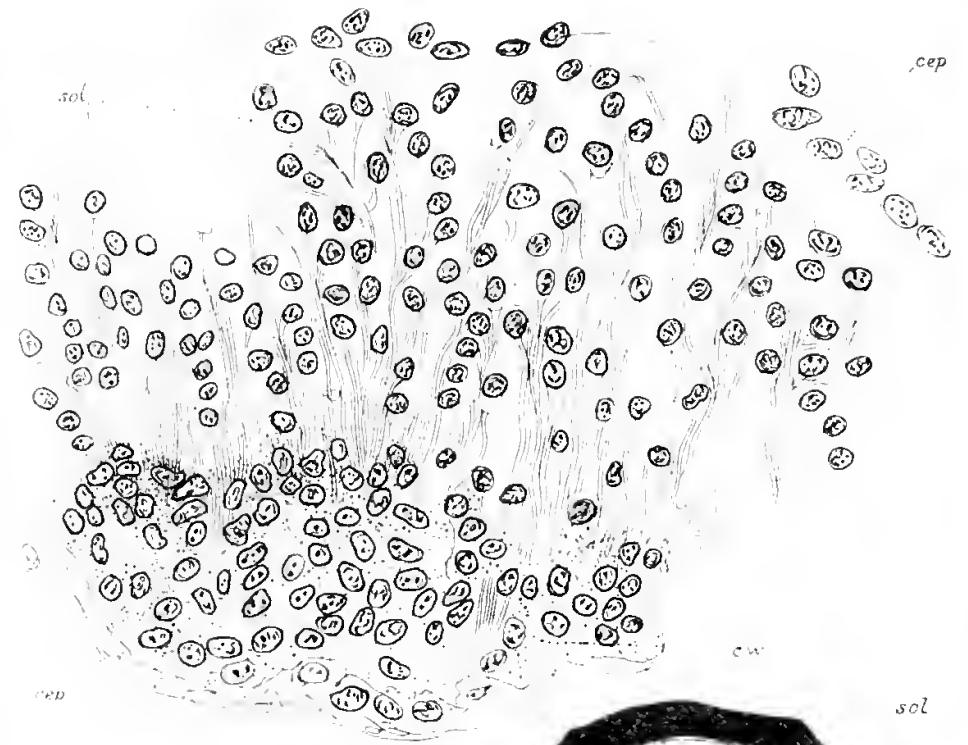
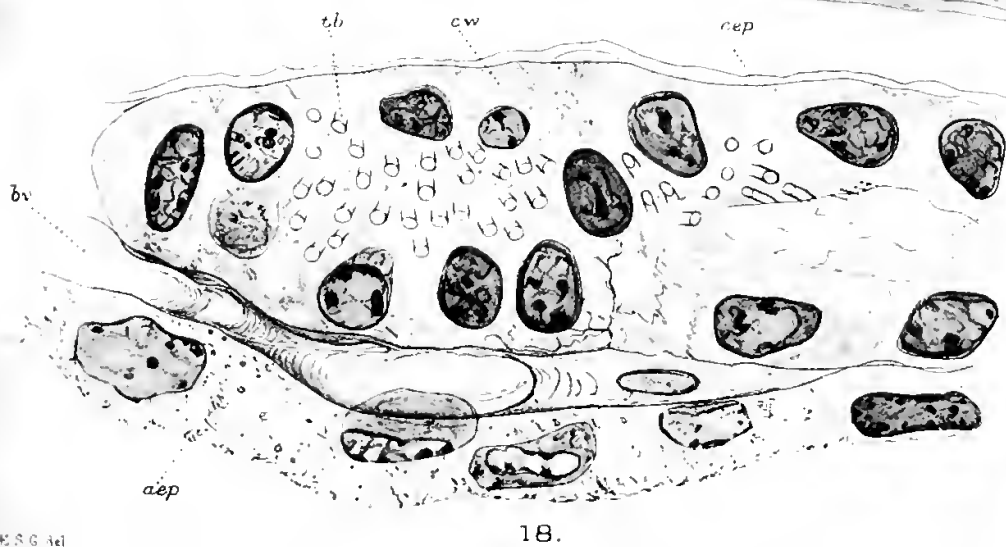
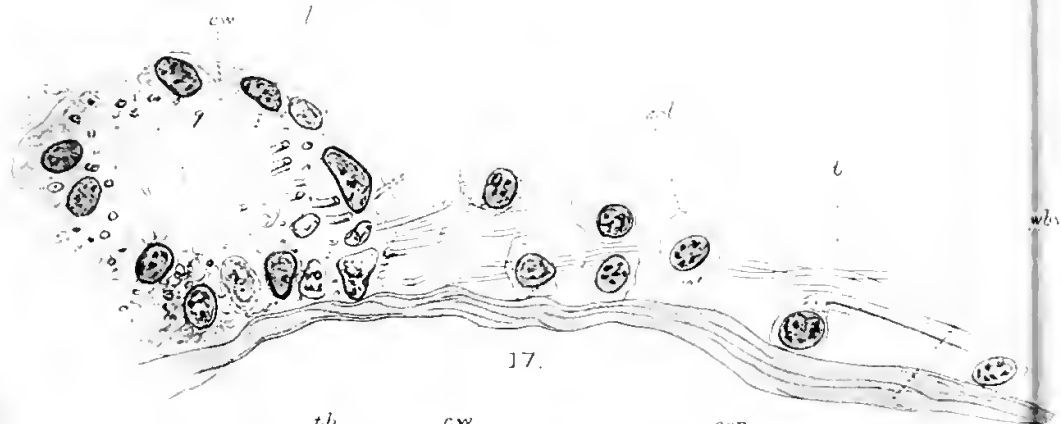
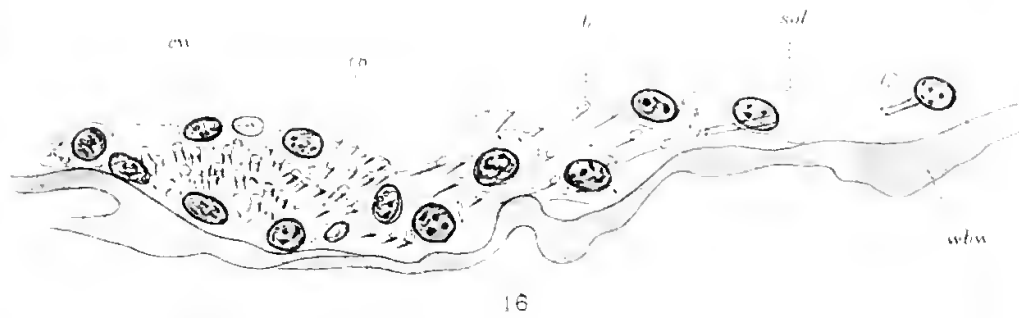
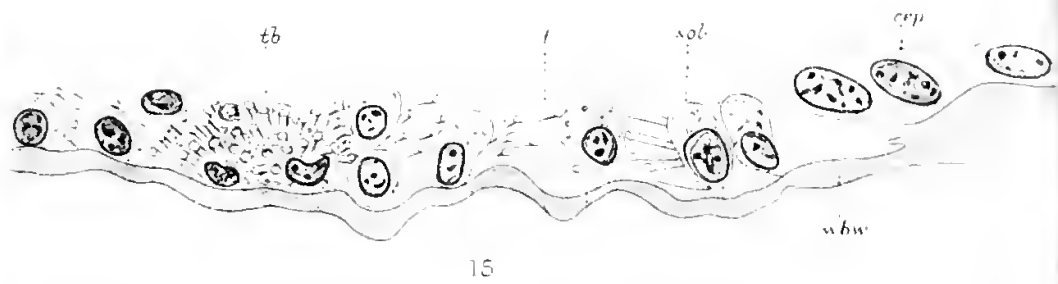








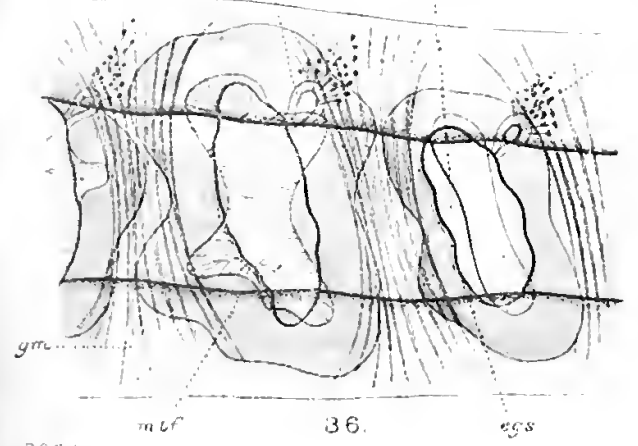
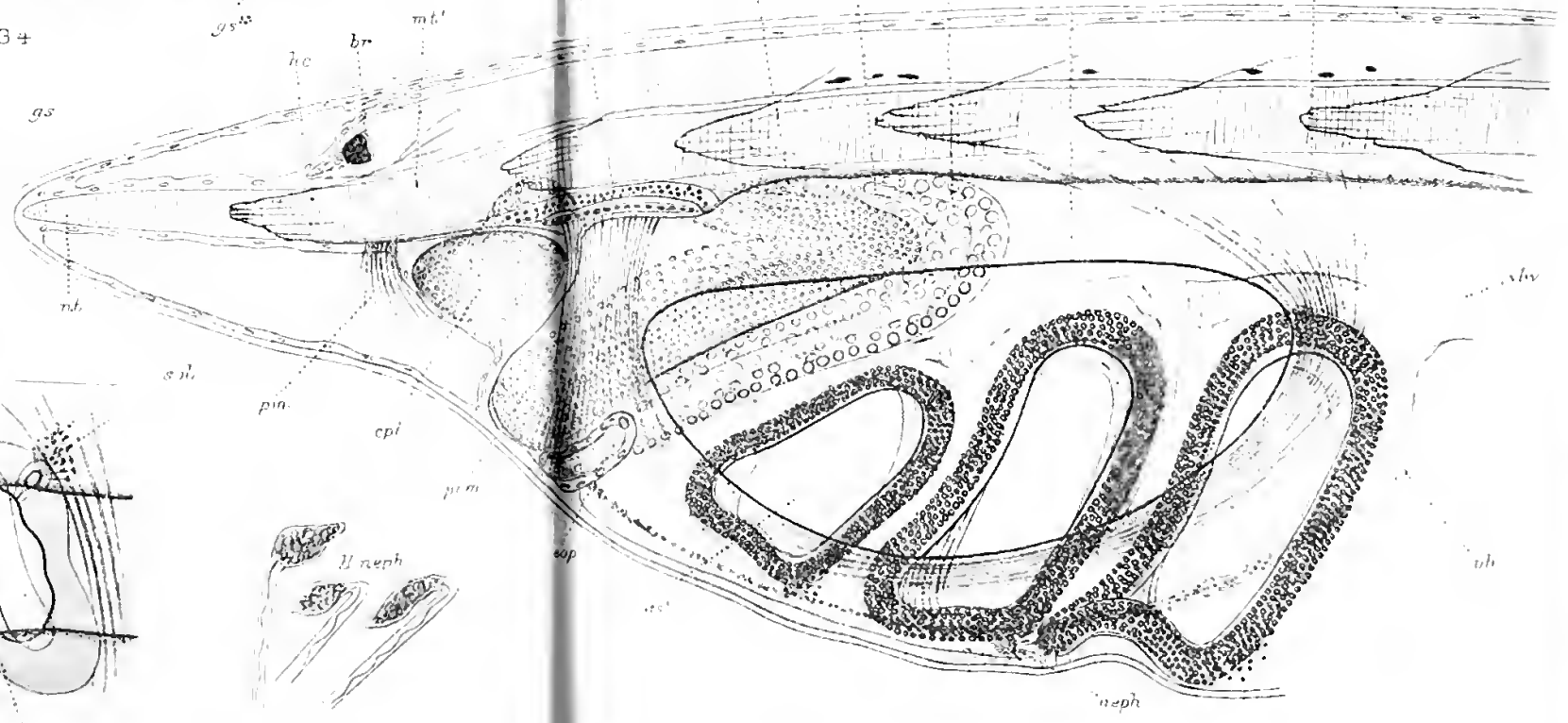
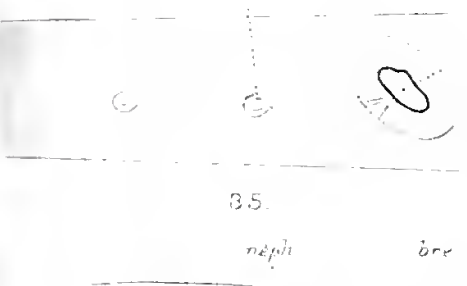
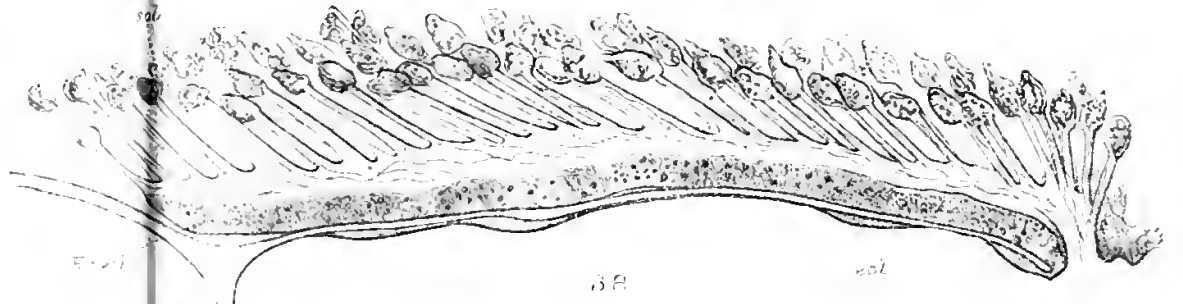
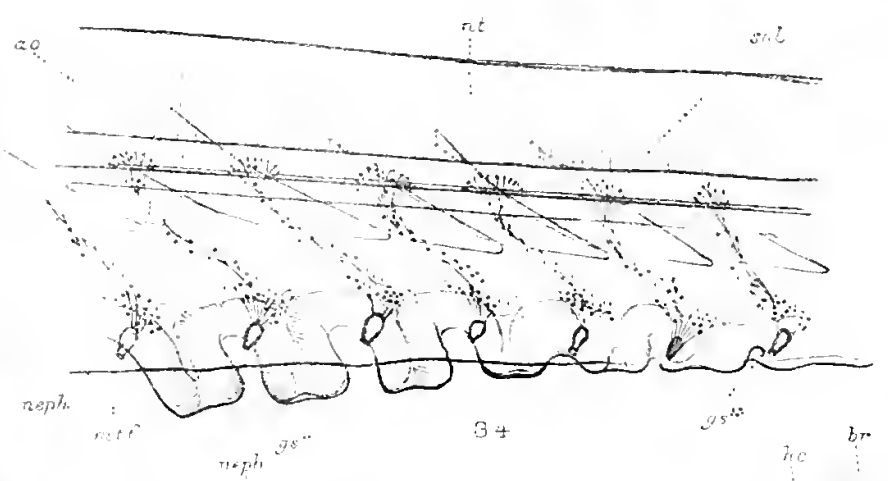
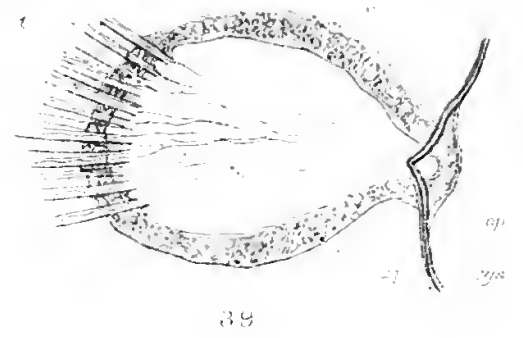
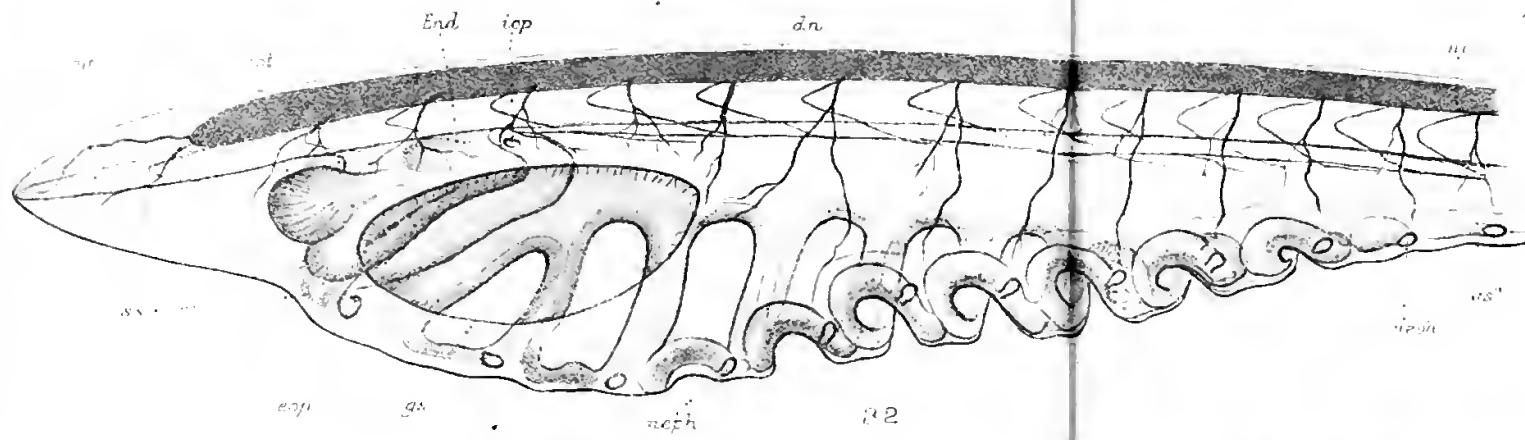


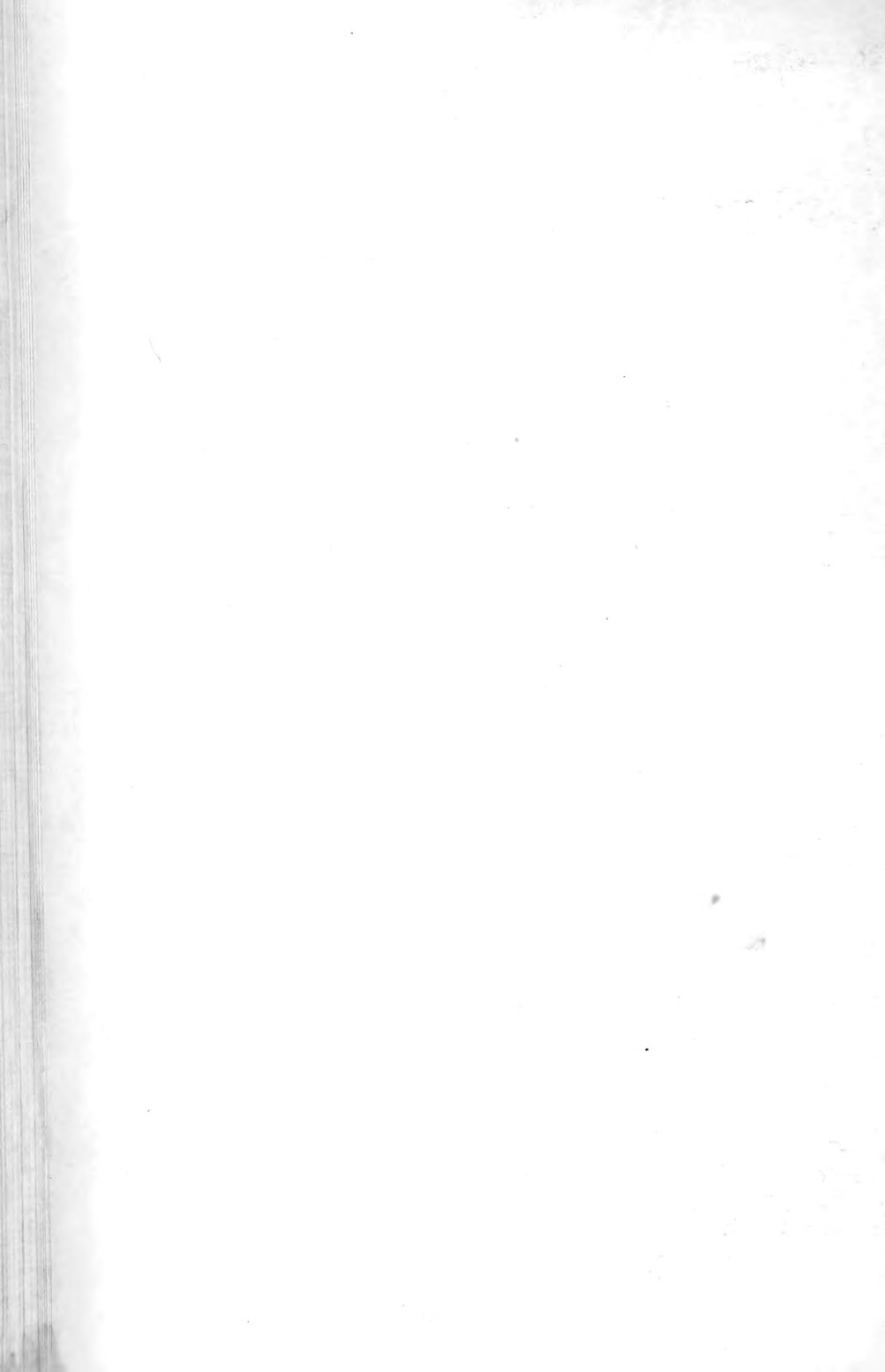


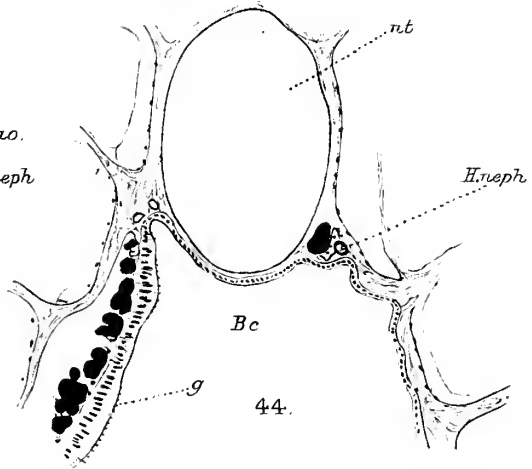
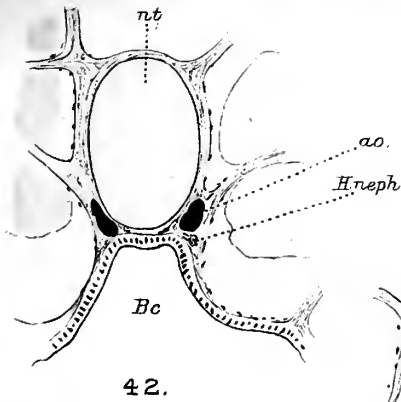






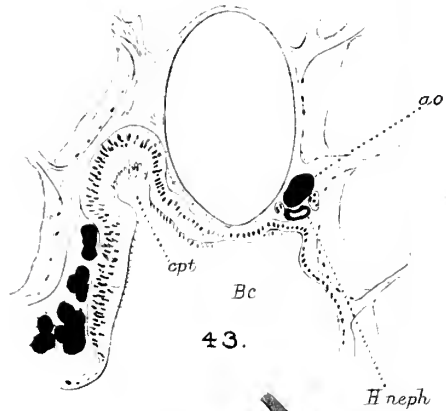
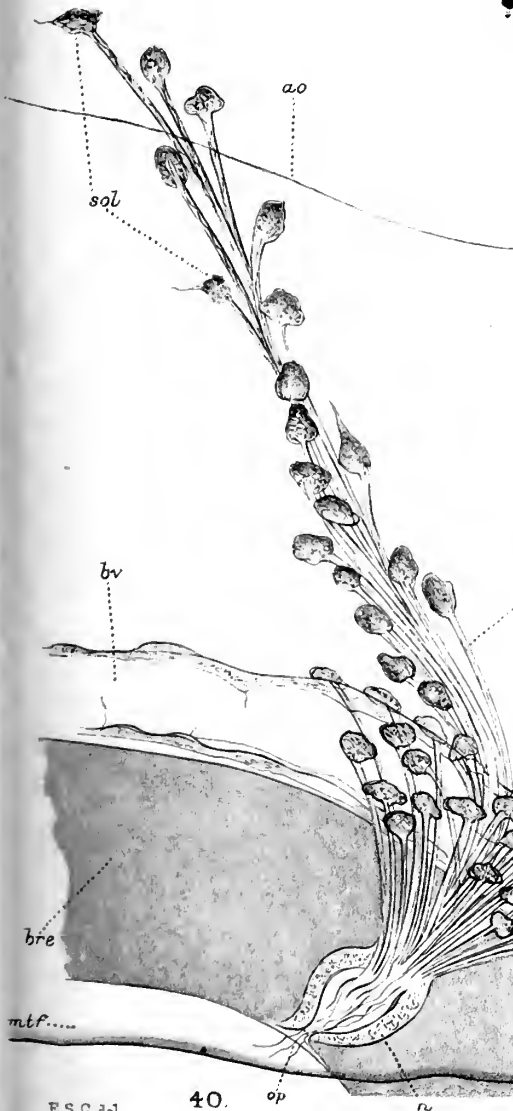




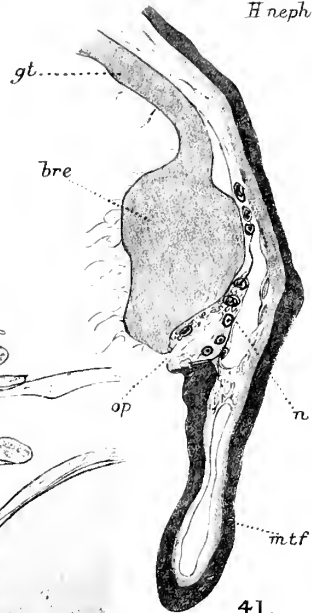


42.

44.



43.



41.

Intra-cellular and General Digestive Processes in Planariæ.

By

G. Arnold.

From the Cytological Laboratory of the University of Liverpool.

With Plate 17.

IN 1878 Metschnikoff drew attention to the phenomena of intra-cellular digestion occurring in Turbellarian worms. Since that time but little has been published dealing with this very interesting subject.

Metschnikoff's short notice was followed by a paper by Lankester dealing with intra-cellular digestion in the endoderm cells in the medusa of *Linnocodium*, and two years later, 1883, Metschnikoff published further observations on intra-cellular digestion in the mesoderm cells of *Synapta* and *Phyllirhoë*.

Intra-cellular digestion has been observed in Cœlenterates generally, sponges, Protozoa, and in the leucocytes of the blood.

Within recent years several observers have dealt with the digestion in the Protozoa, but apparently no work has been published dealing with the cytological details of intra-cellular digestion in any of the Enterocœla.

Mouton in 1902 and Nerinstein in 1905, following on the earlier work of Greenwood and Saunders, have given long and detailed accounts of the process of digestion in *Amœba*, *Paramœcium*, etc. These authors, however, have limited their attention almost entirely to the intimate history and staining reactions of the food vacuoles of those animals, and their conclusions afford few data which shed any light on

the digestion in more highly organised animals such as *Planaria*. Moreover, the methods of research are necessarily different. In unicellular animals a considerable number of facts may be ascertained by the observation of the effects of intra-vitam staining. In animals such as the *Planaria* this is impossible on account of their large size and opacity. The observations here described have therefore been made upon carefully preserved specimens, and the staining reactions are therefore post-mortem.

The methods used were as follows:

A number of *Planaria lactea*, which had been deprived of all food of any sort for fifteen days (after which period of time the cells of the intestine are entirely devoid of all food remains, see fig. 11) were fed with fresh clotted pig's blood, and fixed in Flemming's strong solution at various intervals after feeding.

These intervals after feeding were as follows: $\frac{1}{4}$, $\frac{1}{2}$, $1\frac{1}{4}$, $3\frac{1}{2}$, 27, 48, 52, 70, 76, 96, 118. When a Planarian has just fed, the fixation is attended with difficulty owing to the fact that immediately the animal is immersed in the fixing fluid it contracts and ejects the recently ingested food with considerable violence, not through the pharynx, but anywhere through the skin. If, however, the animal is cut into several pieces at the same time that the fixative is poured upon it, this difficulty is partially obviated, the whole procedure being too rapid to permit of any violent contraction. Forty-eight hours after feeding the lumen of the intestine is almost empty, most of the blood having been ingested, and the *Planaria* fixed after that interval did not eject any of the remaining contents.

The stains used were: (1) A triple stain—Basic fuchsin, methylene blue and orange G.,¹ and (2) iron-alum-haematoxylin, acid fuchsin and orange G.

All the figures, except fig. 5, are drawn from preparations stained by the former process.

¹ I have given an account of the method of using this stain in a paper on the "Ovi- and Spermatogenesis of *Planaria lactea*," 'Arch. f. Zellforschung,' Bd. iii, Heft 3, 1909.

Some cells from the intestine of a planarian which has been without food for some seven or eight days are shown in Pl. 17, fig. 13.

The cells of the intestine are of two sorts:

(1) Long, irregularly columnar cells. The cytoplasm of the cell (fig. 13) consists of a clear protoplasmic network, enclosing several large vacuoles at its distal end, the vacuoles towards the middle of the cell being smaller and fewer. The proximal part of the cell consists of very much denser cytoplasm, in which the reticulum is very fine and close, showing an almost fibrillar structure at its extreme end. The spaces between the network take the acid stain, but the network itself is stained by the basic colours, so that the proximal end of the cell where the reticulum is very dense is much darker than the rest.

In this part lies the nucleus, which is small in proportion to the cytoplasm. The nucleus is round or ovoid, with a deeply staining membrane, and a nucleolus which is stained bright blue by the methylene blue.

In an animal which has been starved for fifteen days the vacuoles in the cytoplasm are more numerous and larger (fig. 11). The cytoplasm around each vacuole is denser and more granular than elsewhere, but a definite membrane cannot be made out.

(2) Goblet-shaped gland-cells, only half as large, or less, than the former, invariably with a small nucleus extremely irregular in outline, and taking the basic fuchsin stain very markedly. The cytoplasm is very granular, and is peculiar in having a greater affinity for basic than acid stains, staining as deeply as the nuclear material. It is full of large vacuoles, in which now and again is to be seen a residue also stained by the methylene blue (figs. 11 and 13.)

ABSORPTION OF FAT.

We will deal with the functions and history of these gland cells first. There is generally one of them to every ten of the

others. When the intestine is empty they are large and the vacuoles are full (fig. 11). Very soon after food has been taken into the intestine the whole cell diminishes in size, till at about the twenty-seven hour stage it is shrunken to a fifth of its original size and quite flaccid (fig. 16). In this condition it lies squeezed in between the columnar cells, so much so that sometimes these cells appear to lie quite outside the intestine, between the latter and the surrounding parenchyma.

There can be little doubt that the gland cells secrete a digestive ferment, which is probably used entirely for the digestion of fat.

Within a quarter of an hour after feeding it will be seen that the columnar cells are full of fat-globules, stained black by the osmic acid of the fixative (fig. 1). Even when the lumen of the intestine is full of blood (red corpuscles, leucocytes, etc.) no fat-globules are to be seen lying free in the lumen, nor can any pseudopodial extensions of the cytoplasm containing fat-globules of the columnar cells be seen, suggesting that the fat has been ingested in an amœboid fashion.

The gland-cells do not begin to return to their normal size till after about the forty-eighth hour, when almost all the columnar cells are devoid of unaltered fat, and reach their usual size again at about the seventieth hour. It is very noticeable that no ingestion of solid particles (i. e. true intra-cellular digestion) takes place until the absorption of fat is over, and the latter has undergone marked changes in the columnar cells. A large part of the fat absorbed by the columnar cells is digested in the cytoplasm of these cells, but some of it is again passed out at their bases unaltered lying in the parenchyma. The fate of these extruded globules will be dealt with later on.

The fat first appears in the cytoplasm of the columnar cells in very small globules, which by fusing together form much larger ones, so that some cells within an hour after feeding seem to be one mass of fat.

The researches of Munk, Moore and Rockwood, and others

have shown that in the higher animals, especially in mammals, the absorption of fat by the epithelial cells of the intestine is brought about by the fat of the food being converted into fatty acids and glycerine by the action of lipolytic enzymes. Only in that form can the fat be taken up by the epithelial cells, which then again synthesise the fatty acids into fat, and the latter is seen in the cytoplasm of the cells in the form of globules, being passed on by them to the lymphatic cells and the lymphatic capillaries.

The process appears to be very similar in the Planariæ, and judging by the facts stated above, there is reason to believe that the goblet-cells of the Planariæ function as organs secreting a lipolytic enzyme. Possibly they may elaborate other secretions as well, but their ability to secrete a fat-digesting fluid can hardly be doubted.

It has been pointed out that when the columnar cells are full of fat-globules stained by the osmic acid, no such fat is to be seen in the lumen. It was therefore necessary to see whether there was any fat in the lumen in a form not acted on by osmic acid. It is well known that the staining with osmic acid is due to the presence of unsaturated compounds. In view of the work of Lorrain Smith ('07) it was thought desirable to test the action of Nile-blue sulphate, which stains not only the neutral fat, but differentiates the fatty acids. For this purpose some Planaria were fixed a quarter of an hour after feeding in a weak solution of formol and cut with a freezing microtome. By this means all fat solvents, such as xylol, etc., were avoided. The sections were then stained for fifteen minutes in a strong aqueous solution of the dye. In spite of the fact that the colour was slightly masked by the blue colour taken by all the tissue, characteristic globules of fat in the columnar cells were seen, red to reddish-yellow in colour. Care has to be taken not to confuse loose red blood-corpuscles which have been shifted from the lumen on to the cells with these globules. The colour is, however, entirely different, the fat-globules being definitely red under a high power lens, whereas the corpuscles

are yellow. Apart from this, fat-globules can be seen in the cytoplasm of the endoderm cells, far too large to be mistaken for any corpuscle lying over or under one of those cells. It must be remembered that no ingestion of the corpuscles takes place until some considerable time after the one hour stage, at which these *Planaria* were killed.

This fact indicates, at least, that the fat which appears in the endoderm cells is a neutral fat, but whether the secretion of the gland cells breaks down the fat of the blood into fatty acids could not be ascertained, for the colour of the blood-corpuscles completely masks any blueness which might be present in the food magma.

However, sections of the one and a half hour stage, cut in paraffin and stained with Nile-blue sulphate, showed a definite bluish tinge in the magma, but not a trace of red.

The significance of this fact is important, for it shows that the digestive process in *Planaria* is not, as has been stated by Metschnikoff ('*L'Immunité*,' 1902), entirely intra-cellular, and at the same time indicates the first step in the formation of the highly complex digestive apparatus found in the higher animals.

This first step is, we have seen, the production of a secretion by certain cells which enables fat to be absorbed. Such cells are unicellular glands. If during the course of evolution these unicellular glands, instead of being diffused throughout the intestine, become aggregated in certain areas, we are enabled to picture the formation of any of the multicellular glands which line the intestinal tract by the subsequent invagination and enlargement.

Metschnikoff ('02) and in his work '*L'Immunité dans les Maladies Infectieuses*,' says that in *Planaria* digestion is entirely intra-cellular, and this seems hitherto to have been widely accepted.

Mesnil ('01) comes to the same conclusion in regard to the *Actinia*, but this disagrees with the results of several other workers.

Pratt's ('05) observations on the digestive organs of the

Alcyonaria, lead her to conclude that large food bodies are rapidly broken up into small particles, and in some cases apparently acted on by some digestive ferment in the cœlenteron of the zooids before being ingested by the cells of the ventral mesenterial filaments, and that "we have evidence in the Alcyonariæ as in the Madreporaria of an intercellular digestion by the secretion of a digestive fluid in the cœlenteron of the zooids, as well as an intra-cellular digestion which occurs throughout the cœlenterates."

Jordan ('07) has come to similar conclusions on the digestion in Actinia, and says that in them digestion is both inter- and intra-cellular. He put little paper bags containing fibrin in the gastric cavity of some Actinia, and found that the contents were digested although the bags remained intact. His results are in agreement with those of Krukenberg.

Even in Hydra, according to Hadži ('06), an appreciable amount of extra-cellular digestion takes place, the food being slightly predigested in the lumen before being ingested by the pseudopodia of the endoderm cells.

We need not be surprised then that in the more highly organised Planarian, digestion is not entirely intra-cellular.

The alteration which the fat-globules undergo in the columnar cells is characterised by very marked alterations in their staining reaction. At first they are deep black owing to the action of the osmic acid in the fixing fluid in which the animals were preserved (figs. 1 and 2).

Each globule is enclosed in a vacuole. Within half an hour after feeding, some of the globules at the free end of the cell become paler, changing from black to grey, and then brown. Within two hours after feeding (figs. 2, 4, and 5) the change had proceeded a great deal further. The black reaction to osmic acid is no longer present, and the fat takes the less basic of the two basic stains, the fuchsin, till eventually it is only stained by the acid cytoplasmic stain, the orange G. (figs. 4, 6, and 7). A vacuole is no longer visible, and eventually the fat-globules are incorporated in the substance of the cytoplasm.

INTRA-CELLULAR DIGESTION.

After all the fat has been absorbed, and when all the gland cells are empty (fig. 10), true intra-cellular digestion (phagocytosis) commences.

The columnar cells push out at their free ends long pseudopodial extensions into the lumen of the intestine, and shortly afterwards large vacuoles appear in which masses of red blood-corpuscles are seen (fig. 3).

At this stage, one and a quarter hours after feeding, the selective action of the columnar cells is very noticeable, for only the red-corpuscles are ingested, but none of the leucocytes. The latter are ingested last of all, some forty-eight hours after feeding (fig. 6).

The digestion of the red corpuscles takes place very slowly. Even ninety-six hours after feeding (fig. 8) they may be seen intact in some vacuoles.

As digestion proceeds, the corpuscles lose their shape (fig. 6, *b.c.*), till at last the vacuoles contain an amorphous mass of particles, consisting chiefly of the envelopes of the corpuscles, which are stained by the methylene blue.

The leucocytes are ingested singly, and a vacuolar space soon appears round them.

The leucocytes which lie in the lumen of the intestine do not appear to undergo any change at all. Even after forty-eight or fifty-two hours they can be seen scattered about in the lumen, their cytoplasm stained orange by the acid stain, and the nuclear membrane and the chromatin in the nucleus quite intact. But immediately a vacuole has formed round them after ingestion (figs. 6 and 7, *L.*) their staining reaction changes. The cytoplasm then takes a pink colour, due to the basic fuchsin, their nuclear contents become diffused, and shortly afterwards the separate chromatin masses are no longer distinguishable (fig. 9).

This marked and rapid change in the staining reaction is undoubtedly due to the fluid in the vacuole secreted by the surrounding cytoplasm.

It is now a generally recognised fact that intra-cellular digestion in Protozoa is accompanied by a secretion of acid in the vacuoles, but with regard to the part played by this acid in the process of digestion there is a large difference of opinion.

Greenwood and Saunders ('94) show that proteolysis commences when the acid reaction is over, and is replaced by a neutral reaction. That the vacuole fluid also contains a proteolytic enzyme there can be no doubt.

Monton ('02) succeeded in extracting from cultures of *Amœbæ* a diastase, chiefly of a proteolytic action and approaching trypsin in its nature. This diastase he identified with the fluid in the interior of the digestive vacuoles.

Nirenstein ('05) does not think that the acid in the vacuoles has anything to do with digestion, and Monton has shown by a most careful series of experiments that the amœbo-diastase which he extracted from *Amœbæ* has a digestive action in an alkaline, neutral or faintly acid medium.

On the other hand, Metschnikoff ('*L'Immunité*'), by feeding *Planaria* with blood with which had been mixed some grains of blue litmus, came to the conclusion that digestion in those animals takes place in an acid medium.

"*L'étude des planaires nous montre que la nourriture des ces animaux subit exclusivement la digestion intra-cellulaire, dans un milieu faiblement acide et avec l'aide d'un ferment soluble. Elle nous fournit déjà une preuve de ce que la digestion intra-cellulaire typique est un processus chimique, dû à l'intervention d'enzymes.*"

I have shown in connection with the absorption of fat that digestion in *Planaria* is not entirely intra-cellular, but the sudden change in the staining reaction of the ingested leucocytes is strong evidence in support of the view that the intra-cellular digestion in these animals takes place in an acid medium. The change in the staining reaction of the cytoplasm of the ingested leucocytes from the normal acid to the basic stains would seem to indicate that the ingested material becomes impregnated by an acid fluid.

Occasionally, in even the earlier stages, some ingested bacteria are seen, but they are not numerous. But in the cells of two *Planaria* killed 118 hours after feeding they were extremely numerous (fig. 10, *b.* and *c.*), and also in the lumen of the intestine.

At this stage the intestine is practically empty, except a few masses of blood-corpuscles and leucocytes, with numerous bacteria. That they appear in greater numbers only when the food, or what is left of it, has been in the intestine for a long period of time would suggest that the remainder of the free food is undergoing putrefaction. No great importance is to be attached to this isolated observation, but perhaps we have here the indication of the formation of a definite intestinal bacterial flora.

CHANGES IN THE NUCLEUS.

In all the columnar cells of the starved examples the nucleus contains only one nucleolus (figs. 11 and 13). At the most active state of digestion (figs. 7 and 10) there are two nucleoli, and sometimes even three. It is a question whether this multiplication of the nucleoli is to be interpreted as an absorption of material from the cytoplasm to the nucleus, or as an expression of increased activity of the nucleus during digestion, with the consequent formation of waste products.

EXCRETORY AND PIGMENT GRANULES.

In all the columnar cells of the intestine certain granular masses are seen. They are highly refractive and preserve a yellow colour independently of the staining (fig. 15). Most of them are excretory products, but some can not be distinguished from the pigment granules which form the greater part of the eyes of these animals. As digestion proceeds they increase in number, but always occur in groups, and are not evenly distributed through the cell.

PASSAGE OF FAT AND EXCRETORY GRANULES INTO THE
PARENCHYMA.

Some of the fat absorbed by the columnar cells is not digested but passed out in globules at their bases into the parenchyma (see fig. 2; on the right a fat-globule is being extruded). These globules are taken up by some amœboid wandering cells (fig. 14), and also by the yolk-cells (fig. 12) and the large parenchyma cells (fig. 17). How these globules reach the interior of the yolk-cells I have not been able to ascertain. Any digestive power of an amœboid nature in the yolk-cells or even in the parenchyma-cells is extremely unlikely. Nevertheless it is very striking that after feeding, the yolk-cells which lie in proximity to the intestine are crowded with fat-globules, whereas in unfed specimens the yolk-cells contain scarcely anything but yolk-globules. After feeding, fat-globules are numerous at the bases of the columnar cells, and lying free in the meshwork of the parenchyma (fig. 14). The parenchyma-cells also contain numerous excretory granules, massed together in vacuoles (fig. 17).

It would be expected that in an animal like *Planaria* devoid of an anus, the excretory products would be shed into the intestine to make their way out to the exterior by the pharynx.

An examination of a very large amount of material, consisting of some hundreds of slides, has afforded no evidence in support of this view. Not only have I been unable to see any extrusion of waste matter into the intestine, but a careful search through numerous sections has failed to show any trace of extruded excreta in the shape of the characteristic yellow concretions in the lumen of the intestine. Are they so soluble that they are all removed when lying free in the intestine by the process of preparing the material for sectioning? If not, it is difficult to explain how they are removed from the body of the Planarian to the exterior.

I wish to express my thanks to Dr. Roaf, of the Department

of Physiology in this University, for valuable advice on staining for fat with Nile blue sulphate.

CONCLUSIONS.

Digestion in *Planaria lactea*, and probably in all Tricladæ, is both inter- and intra-cellular.

The intercellular digestion is limited to fat. The fat is broken down in the lumen of the intestine by the secretion of the goblet-cells into fatty acids, which are then absorbed by the columnar cells and synthesised again into neutral fat.

Most of the fat is digested in the cytoplasm of the columnar cells, but some of it is extruded into the parenchyma at their base, and appears in the yolk-cells and in the wandering cells.

The digestion in the vacuoles takes place in an acid medium, as evidenced by the change in the staining reaction of ingested leucocytes.

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EXPLANATION OF PLATE 17,

Illustrating Mr. G. Arnold's paper on “Intra-cellular and General Digestive Processes in Planariæ.”

All the figures, except 13, are drawn direct, using a 2 mm. oil-immersion Zeiss and 8 compens.-ocular. Figs. 9 and 15 with 18 compens.-ocular. Fig. 13 $\frac{1}{4}$ in. Swift and 6 ocular.

All the figures except 5, which is stained with iron-alum hæmatoxylin-acid fuchsin and orange G., are stained with the triple stain mentioned in the paper.

FIG. 1.—A columnar cell from material fixed $\frac{1}{4}$ hour after feeding.

FIG. 2.— $\frac{1}{2}$

FIG. 3.— $1\frac{1}{4}$ hours

FIG. 4.— $3\frac{1}{2}$

FIG. 5.— $1\frac{1}{4}$

FIG. 6.— 48 . ..

FIG. 7.— 52

FIG. 8.—Portion of a columnar cell fixed 96 hours after feeding, showing pseudopodial ingestion of a leucocyte.

FIG. 9.—Portion of another cell, same stage as 8 (18 ocular).

FIG. 10.—A columnar cell from material fixed 118 hours after feeding.

FIG. 11.—A columnar cell and a gland-cell from an animal starved for fifteen days.

FIG. 12.—A yolk-cell containing fat- and yolk-globules. Yolk coloured blue.

FIG. 13.—Several columnar cells and one gland-cell from a Planarian starved for five days. Normal appearance.

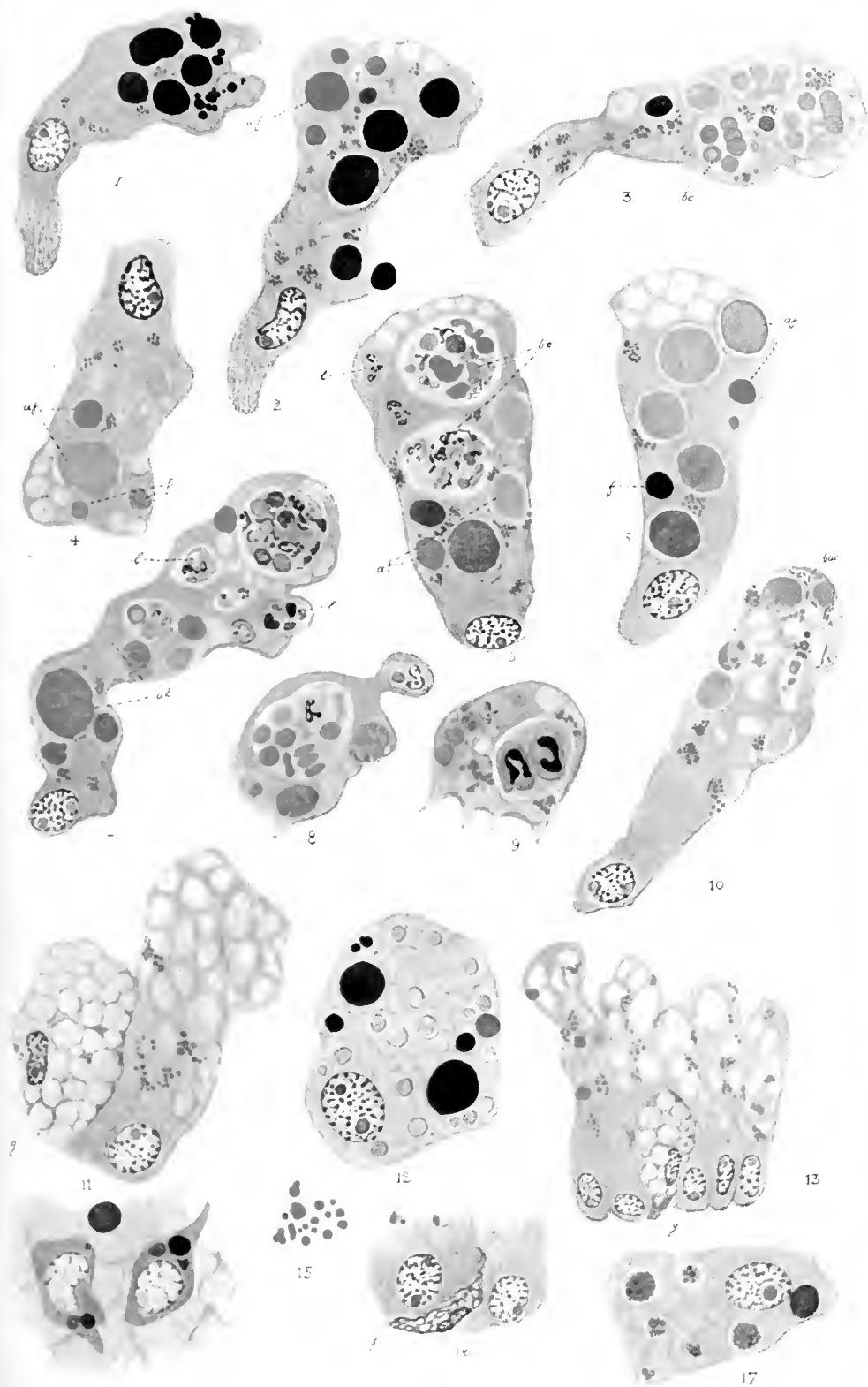
FIG. 14.—Meshwork of the parenchyma, showing a free fat-globule and two amœboid wandering cells, also containing fat, some of which is undergoing alteration.

FIG. 15.—Excretory and pigment granules (18 compens.-ocular).

FIG. 16.—An empty gland-cell lying at the base of two columnar cells, cf. fig. 11.

FIG. 17.—A parenchyma cell containing excretory granules massed together in vacuoles.

f. Osmicated fat. *af.* Fat very much altered and partially absorbed. *l.* Ingested leucocyte. *bc.* Ingested blood-corpuscles. *bac.* Bacteria. *g.* Goblet-shaped gland-cell.





Professor Hubrecht's Paper on the Early Ontogenetic Phenomena in Mammals: An Appreciation and a Criticism.

By

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With 5 Text-figs.

PREFACE.

PROFESSOR HUBRECHT'S paper in a recent number of the 'Quarterly Journal of Microscopical Science' brings together "the results of new investigations and recent reflections with such as had already been published on earlier occasions."

Even if "the new investigations and recent reflections" do not contain a great deal that is new, nevertheless the whole is an invaluable expression of the Professor's present opinion on a subject which he is doing so much to advance and to cause others to devote attention to. At the same time it is not possible to ignore the feeling that this new paper would have been of still greater interest had the author, in addition to the resumé of his results, thought fit to discuss more fully the difficulties which have arisen in the minds of some who are unable to accept his theoretical conceptions.

Hubrecht has no doubt deliberately chosen to leave for the moment unanswered the objections urged against his views, possibly with the hope that objections—if such there are—may be formulated more precisely than heretofore, in which case we may hope for a chapter making good this omission at a no very distant date.

In the hopeful expectancy of such a chapter I venture, as one who has taken a practical part, though but a small part, in the attack upon the problems in question, and as one who appreciates to the utmost the magnitude and inspiring influence of the Professor's work, to urge the force of certain objections which appear to me as formidable obstacles to the acceptance of some of Hubrecht's views.

CHAPTER I.

THE EUTHERIAN BLASTOCYST.

Beginning with the question of the segmentation of the ovum of Eutherian mammals, and passing rapidly over the manner in which the morula stage is produced, Hubrecht describes the embryo of this stage as consisting of an inner mass of cells which he calls the embryonic knob, and an outer layer called the trophoblast, and gives three figures (2, 3, 6, p. 7) in which the inner cells (the embryonic knob) are shown to exhibit "a different reaction against staining reagents than the peripheral" (p. 6), the inner cells being lighter in colour than those of the outer layer.

Perhaps the gist of the whole paper is foreshadowed in the second paragraph of Chapter I, p. 3, where the author speaks of "the erroneous conclusion that the mammalian blastocyst was derived from the Sauropsidan by a process consisting in the gradual disappearance of the yolk, with retention of the other developmental characters."

It is upon the establishment of the erroneous nature of this conclusion that the greater part of the rest of Hubrecht's conclusions must be based.

It seems to me, therefore, to be of the greatest importance to weigh with care the evidence of the manner in which this morula stage of the mammalian segmented egg is attained, and to consider other views which have been advanced, whether from actual observation or as the outcome of reflection.

This part of the development of the mammal Hubrecht treats very cursorily.

[By the way, on p. 4 Hubrecht writes: "There seems to be hardly any doubt that both in *Amphioxus* and in man—the two opposite extremes in the phylum of the Chordata—the two first cleavage cells, if separated from each other, may under favourable conditions each of them develop into a perfect, full-grown individual." Wilson, Morgan, and others have shown that this may be true of *Amphioxus*, but what evidence is there that in man the division that gives rise to homologous twins occurs at this early stage of development? Some years ago I found a case of twinning in the sheep ('98) which I believe is the earliest case known among mammals, and the evidence from that specimen tends to show that the division which results in twinning may occur at a later period, namely, during the formation of blastocyst cavity. I may mention that I recently found in the ferret a condition which at first sight I took to be a similar case; but investigation by sections shows that it is probably not a case of twinning though it may be derived from a bi-ovular follicle after the manner of the pluri-ovular follicles of some *Edentates*.]

On Hubrecht's hypothesis that the trophoblast is derived "from a larval layer, an *Embryonalhülle*" comparable to those of Desor's larva, the *Pilidium*, or the *Sipunculid* larva (p. 17), it is clearly convenient to show that the trophoblast originates by delamination as suggested by the figs. 2, 3, 6, mentioned above, producing a typical *Embryonalhülle* like the hypothetical figure of Hubrecht ('95, fig. *h*, Taf. III), of the originating trophoblast. This also is the way in which arise those superficial layers of anamnia, which Hubrecht subsequently—though, as I hope to show, in some cases quite erroneously—claims as homologous to the mammalian trophoblast.

On the other hand such cases of segmenting mammalian ova as those which supply evidence of the origin of the outer layer by epibole are inconvenient, and this is a point which surely should have been considered very carefully, because it is opposed to the method of formation of the supposed homologous layer of the anamnia, and because it suggests an

entirely different, and, in many respects, more consistent explanation of the origin of the mammalian trophoblast, as has already been urged by other workers on the embryology of mammals.

But what does Hubrecht say with reference to these cases? "The so-called metagastrula stage of mammals, first described by van Beneden ('80), has since been abandoned by that author (though taken up again by Duval ['99, p. 64])." It is quite true that van Beneden has abandoned his explanation offered in 1880 of the epibole, which he described as occurring in the rabbit, but he has not renounced his faith in the fact, but has reiterated it ('99) and described a similar phenomenon in the bat, *V. murinus*, as Duval also has done (Duval, '99). But although Duval supports the metagastrula theory (one, however, which is almost certainly untenable), van Beneden gives a new, and, to my mind, a much more plausible explanation. And at the same time, discussing Hubrecht's theory, van Beneden says of it that "*l'hypothèse de Hubrecht heurte à des difficultés morphologiques et physiologiques insurmontables; elle laisse inexpliquée l'existence, chez les Mammifères placentaires, d'une vésicule ombilicale et d'une foule de caractères communs à tous les Amniotes et distinctifs de ces animaux*" (p. 333).

Hubrecht attempts later to meet some of the objections referred to by van Beneden (also in his former paper, 1902), but he nowhere now discusses the question and significance of the epibole. Does he deny that it may occur?

To me it seems that the evidence in its favour is too strong for the possibility to be ignored. Evidence for its occurrence rests on the observations of van Beneden on the rabbit, van Beneden and Duval on the bat, and myself on the sheep, but it may as well be admitted at once that it is an extremely difficult matter upon which to come to an unhesitating opinion, because in many cases, as, for example, the pig, phenomena which are so strikingly apparent in the bat and sheep are not to be seen at all.

There can be no doubt, however, that in the bat and sheep

and rabbit many specimens show what apparently are stages in epibole with such diagrammatic plainness that the probability of an epibole cannot be ignored, and although such plainness is absent from many, e. g. pig, mouse, dog, etc., there is nothing in these cases which prevents a similar interpretation being placed on them. A difference in staining reaction does not become established until a later stage. In such cases one can neither affirm nor deny epibole, but my point is, there is no evidence against it even in them. In Tupaja, according to Hubrecht, the staining differentiation does not arise until after epibole has occurred. If Tupaja were typical, if the cases of *Lepus*, *Ovis*, *Vespertilio*, were only like Tupaja, then Hubrecht's theory of formation of the trophoblast by delamination might be regarded as established.

I know that in 1894 I myself doubted van Beneden's contention that epibole occurs in the rabbit, but the specimens of segmenting ova of the sheep which I obtained and described in 1898, being extremely well preserved and in excellent condition histologically, were to my mind so convincing that I was quite converted to the view of van Beneden, at least as regards the fact of epibole, although I differed from him in the interpretation of the facts. And since that time we have had the further evidence of Duval and van Beneden derived from their study of Cheiropteran development. If this epibole occurs, that is to say, if there really is a growth of one set of segments round another set during the early stages of the segmentation of the Eutherian mammal's egg, it seems to me possible to hold one of three quite plausible views. Either it is:

(1) An early separation of trophoblast and a growth of trophoblast cells round the embryonic knob;

(2) A growth of the epiblast over the yolk mass like the sliding of the "extra embryonic" epiblastic edge of the blastoderm over the yolk in a bird's egg, as van Beneden suggests (though he does not use the terms "epiblast" and "hypoblast"); or—

(3) A growth over the temporarily lethargic epiblastic mass by the yolk or hypoblast cells (Minot, myself).

The last two interpretations pre-suppose a derivation of the Eutherian mammal from Sauropsidan-like ancestors with large-yolked eggs.

All these are plausible theories, and it would have been very interesting to have had Hubrecht's opinion upon them, especially as the last two are completely opposed to his own views. Incidentally Hubrecht, in connection with the formation of the cavity of the blastocyst, says (p. 6), "E. van Beneden has ascribed the origin of the free space between the epithelial outer layer and the inner mass to the extension of intra-cellular vacuoles ('99). His interpretation has found no support in the results obtained by Keibel and myself, nor in those of Selenka for the opossum." I should like to say that as far as my experience goes the cavity of the blastocyst appears to arise, as van Beneden says, as the extension of intra-cellular vacuoles in the pig and ferret, less clearly so in the sheep (and from general appearance of later stages still less in the goat), while in the rabbit it would seem as distinctly to be intercellular.

Perhaps there is not very much in it, but so far as it goes, if the origin of the cavity is intra-cellular rather than intercellular, it tends towards the probability of the cavity in question being a vacuolation in a yolk bearing syncytium like the germinal wall of the Sauropsidan egg rather than a space between "embryonic" cells and an "Embryonalhülle"; that is to say, it supports the last theory of the three suggested better than either of the other two. There can be no doubt that there are in the sheep, pig, ferret, goat (Assheton, '08, fig. 5), strands of protoplasm which connect the inner lining of the inner mass to the wall of the blastocyst, and this tends to show that the inner lining of the inner mass is of common origin with the wall of the blastocyst; that is to say, the hypoblast and trophoblast are one.

With reference to the three diagrams on pages 229, 231, 233 of my paper referred to above ('08), I fear I have not

made it sufficiently clear that they do not represent any particular animal, but that they are to be regarded as generalised diagrams representing three plausible interpretations of the observed facts of Eutherian early development. The first was suggested by van Beneden's papers on the rabbit and bat. In this I ought to have shown the epiblast thickened at the embryonic pole from the first, because van Beneden lays stress on the fact that the inner mass contains from the first the embryonic epiblast. As drawn it is a compromise between van Beneden's and Duval's account of the bat.

But to return for a moment to the three alternative suggested explanations of the epibole. The first alternative would satisfy Hubrecht's hypothesis so far as the trophoblast of Eutherian mammals is concerned, but how can he accept the explanation if, as he desires to do, he regards this mammalian trophoblast as the homologue of the epidermic layer of epiblast in the Amphibian, or the outer coat and periblast of Teleostean, or certain superficial layers in Sauropsida? In all those cases the layer in question arises later and by delamination, as an investing sheath. There is no hint of a growth round an inner mass. If homologous, is it not strange that the mammalian trophoblast should be formed by epibole? That is to say, Hubrecht cannot well accept this explanation of the epibole, as it would be inconsistent with the rest of his theory.

Other objections to Hubrecht's view are that it does not give a satisfactory explanation of the phenomena known as entypie, nor for the rejection of the trophoblast cells by the epiblast of the embryonal area (pig, rabbit, mole, etc.), whereas if, as the third alternative requires, the trophoblast has had a yolk-mass or hypoblast origin in evolution the rejection is natural enough. Van Beneden's view of the epibole is plausible, but this again does not satisfactorily explain the rejection of the trophoblast cells by the embryonal area, nor does it really explain entypie; and it is not supported by the nature of the epibole suggested by the

segmenting egg of the sheep. Moreover, the growth round would seem to be in the opposite direction to that required by the hypothesis. As I have said on a former occasion ('08), "van Beneden is also a little inconsistent, for in his former papers on the rabbit he shows that the epibole is in the opposite direction to that required by his newer hypothesis. In 1880, in his description of the rabbit, he describes the epibole as occurring in such a way as to place the inner mass at the point where the enveloping rim coalesces (vide van Beneden, '80, fig. 7, fig. 5^{III}), and marks the spot where the embryonal area will eventually be."

The third alternative I still believe to be the most completely consistent explanation of the early stages of the development of Eutherian mammals, and so strongly do I believe in it that I would urge the development of the sheep and bat as strong evidence against Hubrecht's attempt to destroy the old group of Amniota.

This third alternative (p. 226) accounts (1) for the epibole by regarding it as a feature peculiar to Eutherian mammals and due to an overflow, as it were, of the yolk or hypoblast cells over the epiblastic rudiment, which, as the centre whence the great bulk of the animal will be formed (for it includes the whole of the secondary growth centre), is bound to remain inactive.

(2) For this lethargic state of the epiblastic mass continuing through many days, until the space necessary for development of the embryo, on the old Sauropsidan egg type, has been provided.

(3) For the sharply marked off character of the epiblastic mass.

(4) For the frequent protoplasmic connections between the inner layer of the inner cell mass and the blastocyst wall.

(5) For the fact that cells of the inner mass pass into the outer cell wall (Assheton, 1908).

(6) For the rejection by the embryonal area of cells of the trophoblast layer.

Lastly, it may be said that the theory demands no change

of function, the hypoblast or yolk-cells having retained their function of providing nourishment for the developing embryo throughout the entire period of transition from meroblastic to holoblastic conditions.

But of course it involves descent of mammals from large yolked eggs of the Sauropsidan type, and is, therefore, so diametrically opposed to Hubrecht's hypothesis that he would seem to consider it unworthy of consideration.

2. THE METATHERIAN AND PROTOTHERIAN BLASTOCYST.

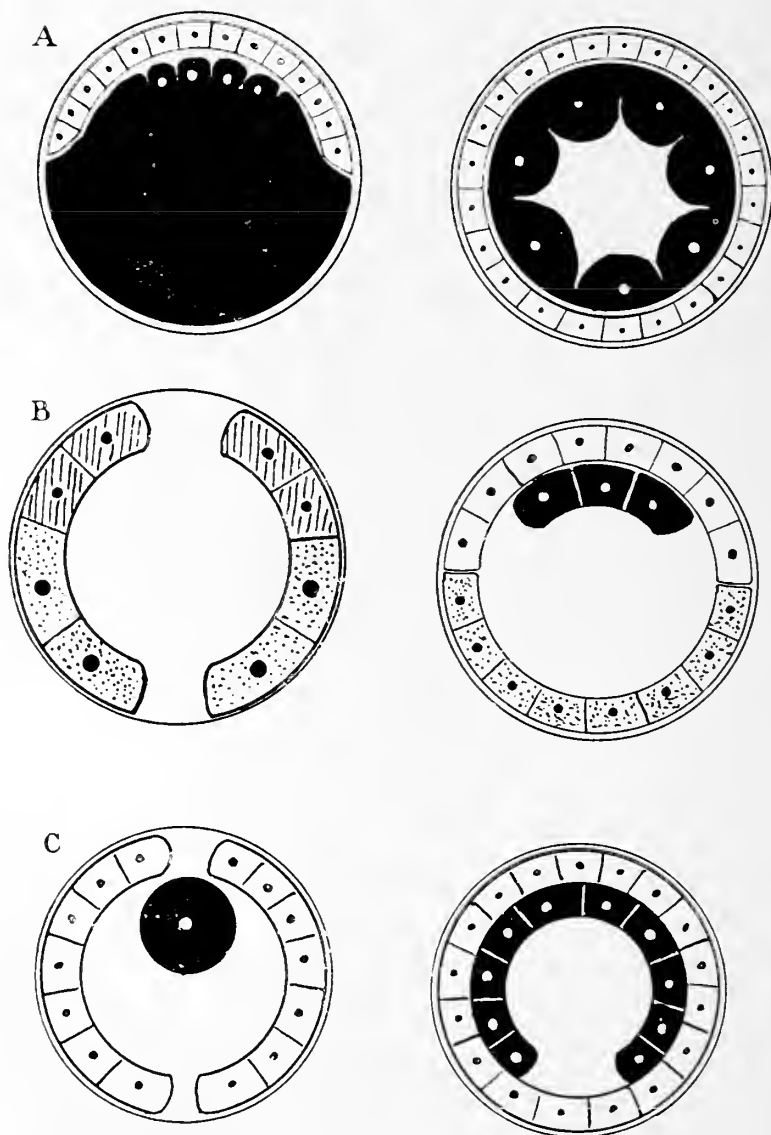
Hubrecht terminates the above discussion with the conclusion that "all the Didelphia and Monodelphia hitherto investigated show at a very early moment the didermic stage out of which the embryo will be built up enclosed in a cellular vesicle (the trophoblast), of which no particle ever enters into the embryonic organisation." Leave out the words "Didelphia and," and agreement with the conclusion will be easy enough.

The only cases we have recorded of the earliest stages of the Didelphia are that of the opossum by Selenka ('87), and those of *Dasyurus* and *Perameles* by Hill ('08). Hubrecht's interpretation of Selenka's figures is well known from his paper on *Tarsius* ('02), in which (p. 55 et seq.) he argues that the outer layer of the vesicle, Fig. 10, Taf. xvii of Selenka's paper, is trophoblast, and that the large inner cell, *en.*, gives rise to the whole embryonic ectoderm and endoderm.

In the accompanying text-figure, fig. C represents Selenka's description of the opossum blastocyst.

Hubrecht's interpretation would be legitimate enough if we regard the character of the cells as drawn by Selenka as only diagrammatic. If, however, Selenka's sections are accurately represented, it is very hard after studying Selenka's figures 2, 3, 4, 8 on Taf. xviii to believe that the thickened outer part of the outer layer labelled *ex* in fig. 4 has really been derived from the group labelled *en* in fig. 2

TEXT-FIG. 1.



Diagrams to show the formation of the blastocyst in: A, Prototheria after Caldwell, Semon, and Hill and Wilson's description; B, Metatheria after Hill's description of *Dasyurus*; C, Metatheria, after Selenka's description of the opossum. White = epiblast; black = hypoblast and yolk; dotted area = trophoblast; shaded area = embryonic knob.

in fig. 10, Taf. xvii. When we turn to *Dasyurus* there is still greater difficulty. According to Hill's ('08) interpretation of his specimens the descendant cells of the lower of his two primary rings which result from the segmentation of the ovum are to be regarded as "trophoblast," the descendants of the upper ring as epiblast and hypoblast as indicated in my diagram, text-fig. 1 B.

If this is the correct interpretation clearly there is no time when the conditions are as all agree them to be in Eutherian mammals.

There are certain fundamental differences between the conditions of the segmenting Metatherian and Eutherian eggs—if the only two cases so far known of the former are typical of the group. Thus, in the Metatherian egg in the four-segment stage the segments are like those of *Amphioxus* or frog, in one plane, whilst in the Eutherian egg they are always eventually in pairs lying across one another, as R. Hertwig points out in his article in O. Hertwig's 'Handbuch,' vol. i. With this is probably correlated another great difference, namely, that the result of further segmentation is in the Metatheria a hollow blastula, in Eutheria a solid morula. Whether Hill is right in calling the lower ring ectoblastic trophoblast is perhaps doubtful, but clearly there is no hint in his description ('Nature,' '08, p. 649) of any Embryonalhülle, any layer lying over the definitive epiblast, at any time of segmentation. Hill has not yet published any detailed account of the formation of the hypoblast; but there is nothing in what he has published to prevent one regarding the trophoblast as of yolk-cell or hypoblastic origin. The difference, then, between the Metatheria and the Eutheria would in that case be that while in the latter the hypoblast and yolk-cell mass overflow the embryonal area and give rise to the "Rauber layer," to use an old term, in the Metatheria it never overflows. So in the Metatheria there is neither Rauber layer nor entypie. The condition of the opossum is less easy to bring into line with my view unless one takes it to have been brought about by a diminution of

the whole yolk and hypoblast mass, so that the large inner cell, called by Selenka hypoblast, corresponds to the lower ring of Hill's description and has slipped inside the upper ring, resulting in a condition not unlike the Monotremes (vide diagram A in the above text-figure).

I should like to take this opportunity of correcting an error in a drawing on p. 681 of O. Hertwig's 'Handbuch,' vol. i. Fig. 244 is said, by Professor Richard Hertwig, to be "Furchungsstadien des Schafes nach Assheton," and in it two figures are given of what appears to be the four-cell stage of the sheep. The drawings are really those of the rabbit, not sheep, and are taken from my '94 paper on the "Re-investigation into the Early Stages of the Development of the Rabbit."

The second figure shows the four-celled stage with the segments arranged in the way I have just taken to be typical for Metatheria. I have no recollection of the specimen now, so cannot say whether the manipulation can have in any way accounted for the abnormal displacement—if abnormal it is, as I believe.

Anyhow, it is the only figure I have been able to find after searching Bischoff, Coste, van Beneden, Duval, Selenka, Sobotta, Heape, Melissinos, etc., who have observed specimens of Eutherian four-segment stages in which the segments are arranged thus.

A possible—and indeed probable—explanation is that this specimen was obtained immediately after division, at which time it is possible that they may lie in one plane, the twisting being due to some cytotropic influence which places the two pairs almost immediately in the crossways position, where they remain for the next two or three hours till the third cleavage plane arises. It is clear that whereas it may very rarely happen that the stage immediately succeeding the second division is obtained if it last but a few minutes, the four-celled stage in the crossways position lasting two to three hours will turn up much more frequently. Of recent years I have seen four-segment stages of rabbit, pig, dog, ferret,

and hedgehog, and I have always found the pairs cross-ways.

On the other hand, the fact that a three-segment stage is often met with suggests that one segment as a rule divides before the other segment, and so brings about the cross-ways position.

Discussing the arching of the epiblast plate while bursting its way through the trophoblast as it does in *Tupaja*, *Talpa*, *Ovis*, *Sus*, etc., Hubrecht says: "The causes of the folded condition of the embryonic shield can hardly be so simply mechanical as Selenka supposed. They remain obscure for the present, and will come anew under consideration when the origin of the amnion will be discussed" (p. 10). It is difficult to believe that the arching does not aid in the rupture of the trophoblast, though the fact that it brings about the rupture—if it is a fact—is not necessarily the reason why the arching occurs, because the rupture is brought about by other means in cases like *Lepus* or *Sorex*.

The causes are less obscure on the hypothesis that Eutheria derive their peculiar conditions from Sauropsidan ancestry.

With reference to the Monotremes Hubrecht speculates as follows. He admits that "our knowledge is as yet very scanty," but brings what is known about the segmentation into line with the higher mammals by homologising the outer layer (which Wilson and Hill regard as epiblast) with the trophoblast of the Eutherian, and Wilson and Hill's (also Semon's and Caldwell's) entoderm with the embryonic knob of Eutheria, and regards the yolk as an accumulation on an ancestral type peculiar to Prototheria and not derived from Sauropsida.

For this view I can see no reason derivable from actual specimens described and figured by those four authors. Hubrecht's fig. 67, p. 23, might be that of a Sauropsidan, e.g. sparrow; nor do 68, 67, 66, really differ from the Sauropsidan blastoderm. Possibly the segments in 66 look rather less part of the yolk than is often the case in Sauropsidan eggs, but it is certainly remarkable that there should be the

usual plug of fine yolk (characteristic of the Avine egg as the nucleus of Pander) under the segmented area if the Monotreme egg is to be regarded as a trophoblastic vesicle, including "besides an embryonic knob a very considerable amount of food yolk, the development of which will have gone parallel with the change in the ancestral line from viviparity to oviparity."

There is no trace of a breaking through the trophoblast by the inner cells, which, on the contrary, seem to spread out under the outer layer into a thin membrane. When at a later period the primitive streak proliferating area seems to project through (Wilson and Hill, Pl. 3, fig. 26) after the manner of Selenka's figure of the mesoblast pushing through the epiblast in fig. 9, Taf. xviii, of the opossum, this condition could not be taken as the pushing of epiblast through a trophoblast, as the neural plate is undoubtedly in front of this area, and has been formed from the originally outer layer.

So that neither in the Prototheria or the Metatheria is there any really tangible evidence of a trophoblast occurring as a covering layer over the definitive epiblast as in Eutheria.

Summarising up to the present stage I submit that Hubrecht, while ignoring alternative interpretations, has not made good his own case either for the presence of the trophoblast layer in Prototheria and Metatheria, or for the origin of the trophoblast in Eutheria, as a special Embryonalhülle formed by delamination from the epiblast, and has not attempted to meet any of the objections presented to his theory by the study of the segmenting stage of such mammalian eggs as those of *Lepus*, *Ovis*, and *Vespertilio*.

PHYLOGENETIC ORIGIN OF TROPHOBLAST.

Confirmed in an opinion which as regards Prototheria and Metatheria is based on very doubtful evidence, that all classes of mammals have a larval envelope, the trophoblast, Hubrecht proceeds to speculate upon its origin outside the group, and starting with a cœlenterate ancestor, says: "A tendency to

exchange the radial for a bilateral symmetry and to separate the coelom from the enteron must at one time have characterised certain coelenterate ancestral forms, as has already been advocated by Sedgwick ('84) and by myself ('05) on earlier occasions. It is not straining the imagination to assume that in this line of descent closely related forms may have developed, some with, others without, a larval envelope, temporarily ensheathing the cellular elements that will build up the embryo itself, and thus foreshadowing the separation among their later vertebrate descendants of such with and such others without a trophoblast." He then shows how this sporadic appearance of larval envelopes occurs in Nemerteans and Annelids. Why, therefore, not also in Vertebrata?

On the assumption of a terrestrial life an animal "would doubtlessly score certain advantages if at the same time it became viviparous. . . . And towards the efficiency of this viviparous condition the larval envelope could immediately contribute by the mere change of its protective or locomotor significance into an adhesive one" (p. 18), and thus we are led on to the conception of the origin of the mammalian placenta.

Hubrecht quotes Mehnert as showing the existence of an outer or trophoblastic layer in Sauropsida, e. g. tortoise, lizard, and snakes and many birds. But Hubrecht himself finds that Mehnert is proving too much, and rejects those cases which are inconvenient, but retains one case, that of *Clemmys*, described by Mitsukuri, and also *Sphenodon* and *Chamaeleo*, on the authority of Schaninsland, as being truly trophoblastic, and dismisses the rejected ones as cases "distantly comparable to a mammalian epitrichial layer." Of course on the theory I advocate, the trophoblast is of Eutherian mammalian origin only, and is not homologous to any form of envelope outside the group of Eutherian mammals.

Turning to the Ichthyopsida Hubrecht finds the Embryonalhülle present as the Deckschicht in Amphibia and Ganoids and Dipnoi, and "more unquestionably" in the Teleosts.

As regards the Teleosts I have myself ('08), in my paper on

the "Teleostean Eggs and Larvæ from the Gambia River," shown how like the conditions are, that is to say physiologically, to the mammalian egg, for the Deckschicht is continuous with the yolk mass, which is one piece with the hypoblast; but I see no reason to think this is anything but analogy.

The Deckschicht of the Amphibia is, however, a very different thing. It was to the Deckschicht of Amphibia that Hubrecht in his paper ('95) drew attention as being the homologue of the trophoblast and amnion of mammals. But as he says now, "I would never look upon the Deckschicht of the Amphibia as having been the first starting-point of what afterwards becomes amnion and chorion of the higher mammals. We may safely say that Deckschicht and trophoblast are homologous and of similar descent, but we cannot at present fully picture to ourselves what has been the arrangement of the larval envelope in the common parent form from which both have derived" (p. 81).

Again, "the cells of the Deckschicht proclaim their transitory and larval significance yet further by the fact that they disappear in later developmental stages, and that it is only with the constitution of peculiar larval organs that they play any part" (p. 80).

"It should, however, be observed that if we are willing to admit the homology of the Amphibian Deckschicht with the Mammalian trophoblast, we must then unhesitatingly go one step further" (p. 81). Thus, Hubrecht clearly relies chiefly upon the assumed homology of the Amphibian Deckschicht and Mammalian trophoblast.

I think I can convince Hubrecht that the Amphibian Deckschicht must, like the "epitrichial layers" of the Sanropsids, be rejected as something different from a trophoblast or purely larval envelope.

If Hubrecht will examine sections of the segmented egg of *Rana temporaria*, *Bufo vulgaris* and probably other Anura and of larval stages up to 5 or 6 mm., he will find that the Deckschicht layer takes a very important part in the formation of the tissues of the brain. They become the neuroglia

cells of the brain and spinal cord, while the "Grundschiebt" becomes the nervous tissue. It is, in fact, a separation into what His termed "spongioblastic" and "neuroblastic" tissue.

The early stages of this process are described in a paper by myself with figures in vol. 37 of this Journal, pp. 166-169 and Pl. 18.

To summarise the present section I would urge that Hubrecht has not established the homology of the envelopes he mentions either among themselves or to the trophoblast of Eutherian mammals, because:

(1) The epidermic layer of the Anura, upon which great reliance is laid for the support of the theory (p. 81), is neither morphologically nor physiologically similar to the trophoblast of Eutherian mammals, but is in fact the result of an early separation of spongioblastic from neuroblastic elements of the ectoderm.

(2) The vestiges of an outer envelope which Hubrecht retains as instances of larval envelopes among the Sauropsida differ from those he rejects as epitrichial only in the time of their development. The former appear before amnion formation, the latter after. Quite similar vestiges may be found among the Mammalia which render the comparison unconvincing, because the real trophoblast in the Mammalia is obviously present elsewhere.

It is curious that Dendy ('99) should make no mention of the occurrence of this layer in Sphenodon. But if we take Schauinsland's figures as being correct and regard the presence of this layer as a well-established fact, we cannot compare it with the *deckschicht* of Anura because it takes no part in the formation of the embryo, nor with the Teleostean layer because it is quite free from the yolk-mass.

A comparison with the Eutherian trophoblast is hardly less difficult. Hubrecht attempts to do so by comparing the whole extra-embryonic epiblast of Sauropsida with the trophoblast of Eutherian mammals by supposing the double-layered condition of, for instance, Chameleo (Schauinsland, '03, figs. 182-219) to be a separation into cyto-trophoblast

and plasmodi-trophoblast. But where, even in *Erinaceus* or *Vespertilio* which he cites, can a plasmodiblast layer be found as a continuous thin layer of squamous cells? The character of the layer as drawn by Schauinsland is that of an ordinary superficial layer such as may be found on the embryo themselves of *Sauropsida* and *Mammalia* alike.

Nevertheless pages 19-26 are by no means the least interesting of Hubrecht's stimulating work.

(3) The comparison of the outer layer of the blastocyst of *Prototheria* and *Metatheria* (which is admittedly developed in a very different manner) to the trophoblast of *Eutheria* seems to me, with all respect to his great authority, to be based on the slenderest of foundations.

This leaves us with the traces of larval envelope found in *Dipnoi* and *Ganoids* and *Teleosteans*. There is certainly in *Dipnoi* a separation into the layers of epiblast, though not so distinct a one as in the *Anura*, and its subsequent history is not like that of the *Anura* with reference to the central nervous system.

Graham Kerr (1903) (Pl. 4, fig. 18, '*Quart. Journ. Micr. Sci.*,' vol. 43), shows a neural groove into which the outer layer of epiblast passes, but the groove is very shallow, and although a chink is present for a short time (fig. 19) in which some of the outer layer cells might be imbedded, it is not at all probable that the outer layer takes any part in the formation of the central nervous system. Graham Kerr says: "The whole thickening of the keel is confined to the deep layer of the ectoderm—the outer layer passing unaffected over the floor of the groove."

But neither in *Ceratodus* nor *Lepidosiren* does the distinction seem to be of the same nature as in *Teleostei*.

In the *Teleostean* the condition is far more like that of the trophoblast of the *Eutherian* mammals than is any other of the supposed larval layers mentioned by Hubrecht. It clearly is not concerned structurally in the embryo formation, and being continuous with the periblast—indeed part of the periblast—it may be said to be trophic. Also it separates off

at an extremely early age (Kopsch, '01, Assheton, '08), thus resembling the Eutherian trophoblast.

The Teleostean Deckschicht differs from those of the Amphibia and Dipnoi in being quite free from any connection with the lips of the blastopore, over which it passes as a continuous layer, e. g. *Gymnarchus*, *Salmo*.

In view of the uncertainty of its presence in Sauropsida, of its widely differing character and relations among Amphibia, Teleostomi and Dipnoi, of its unique character and function in Eutherian mammals, of the dubious nature of its existence in Monotremes and Marsupials, is it not rash to regard all these outer layers as homologous, and as constituting a feature of such importance as to justify the abandonment of the old group Amniota and the formation of a totally new association, having this feature as the chief diagnostic character? Anyhow, the points to which I have drawn attention seem to me to deserve further consideration.

Another point I might make here. On p. 106 Hubrecht writes, with reference to his very remarkable observations published in 1899 on the blood formation in the *Tarsius* placenta: "The production of blood-corpuscles by the cells of a larval envelope is surely an unexpected histological phenomenon. Still, the details of differential segregation during the successive stages of cell lineage are not yet well enough known to justify any apodictic negation. The possibility is not excluded that at the first cleavage (suppose this to separate trophoblast from embryonic knob) certain potentialities of hæmatogenesis may be passed on to this trophoblast mother cell."

On the hypothesis that the Eutherian trophoblast is really of yolk-cell or hypoblast origin, the formation of blood-corpuscles from it is much less extraordinary, for it is from this layer that the first formed blood-cells arise in Sauropsidan and other vertebrate embryos.

PROTOGENESIS OR KEPHALOGENESIS?

DEUTEROGENESIS OR NOTOGENESIS?

I hope I have a desire no greater than is legitimate to support my own view of processes embryological.

I should, however, like to state clearly why I persist in using the terms "protogenesis" and "deuterogenesis" in preference to the terms coined by Professor Hubrecht, namely, "Kephalogenesis" and "Notogenesis," which obviously refer to the same phenomena.

I do so because Hubrecht's words express conceptions, which, although having reference to the same phenomena which I wish to express by protogenesis and deuterogenesis, signify a different interpretation, which, in my opinion, does not represent the actual facts. And I would even claim some consideration because I believe that I was the first to recognise that protogenesis is in essence the production of a radial symmetry due to growth from one centre involving gastrulation, and that deuterogenesis is growth in length, bringing about bilateral symmetry and has nothing to do with gastrulation (though it may be an inevitable consequence of it), which conceptions I expressed at an earlier date under the terms primary and secondary growth centres (1894). Protogenesis and deuterogenesis form a convenient paraphrase of those terms.

On p. 63 Hubrecht claims to have been with Keibel the godfather of the unwarranted hypothesis that gastrulation occurs in two phases (Keibel, '89, Hubrecht, '88).

Who claims actual parentage I do not know. But that it was a most mischievous and awkward child I can well believe.

Fortunately both godfathers have fiantly disclaimed, by their papers in the year 1905 ('Anat. Anz.,' 'Quart. Journ. Micr. Sci.'), any further responsibility in their adopted offspring, and they now admit that it is a matter of great importance in vertebrate embryology to distinguish between

the true gastrula stage and the post gastrula stage, which latter is the growth in length of the embryo, and it is because I also believe so strongly in the importance of the distinction that I wish to establish the more accurate terms above. I may also claim to have arrived at my conclusion by actual experimental observation, having spent much time in so doing, and, as evidence of this, I may mention my papers of the years 1894, 1896, 1905.

Hubrecht hardly does me justice in ignoring my experimental evidence on the subject. Moreover, this theme was the gist of my three papers in 1894, "Re-investigation of the Early Stages of the Development of the Rabbit," "The Primitive Streak of the Rabbit," and "On the Growth in Length of the Frog Embryo," from the last of which I may quote one paragraph from p. 238: "In other words, I believe that as in the rabbit, so in the frog, there is evidence to show that the embryo is derived from two definite centres of growth, the first, and phylogenetically the older, being a protoplasmic activity which gives rise to the anterior end of the embryo (= gastrula stage); the second, which gives rise to the growth in length of the embryo; which centres of growth occupy the same relative positions in location and in sequence of time, and probably to each are due the same parts of the embryo."

In my subsequent papers on the chick, 1896, and growth centres, 1905, I have described some of a good deal of experimental work which I have done in confirmation of this opinion.

But although Hubrecht now quite agrees that notogenesis has nothing whatsoever to do with gastrulation, it is quite evident that his conception of notogenesis is not the same as mine of deuterogenesis, and since I believe that my conception is nearer the truth than his, I must explain where, as it seems to me, his error lies.

In the first place I should like to refer to a footnote which appears in Hubrecht's English edition of his paper, "The Gastrulation of Vertebrates," in the 'Quart. Journ. Micr.

Sci.,' vol. 49, in which he says that I quite misunderstood his German version, in so far as I believed him to "hold the vertebrate mouth to be in any way derived from the stomodæum of an Actinia-like animal." I am sorry I made the mistake, which I made at least by implication, confounding in my mind Hubrecht's with Sedgwick's very similar theory, published in 1884, to which, by the way, Hubrecht made no reference when he put his forward in 1902.

The accompanying text-fig. 2 shows clearly enough what is the difference between Hubrecht's conception and mine, and why I prefer protogenesis and deutero-genesis to cephalogenesis and notogenesis.

The aboral surface of the cœlenterate, according to Hubrecht, becomes the ventral surface of a vertebrate; according to my interpretation the aboral surface becomes the anterior. According to Hubrecht the oral surface of the cœlenterate becomes the dorsal surface of the vertebrate; according to me the oral surface of the cœlenterate is the posterior surface of the vertebrate, and so on, as shown in the figure.

I claim that my interpretation is founded upon actual experiment on the living embryo, which can be tested by anyone.

Where can Hubrecht find experimental evidence in support of the elongation of an actinian or other mouth in, for instance, the frog, with concrescence or coalescence of its walls? Or how can a theory of concrescence be reconciled with experiments such as those of Kopsch on the trout?

I claim that the experiments which I described in my paper of 1905 prove that in the frog at any rate the embryo does grow in the way illustrated by my figures, and that this is absolutely opposed to the method of growth required by Hubrecht's theory.

Again, with reference to notogenesis, Hubrecht seems to have no very clear conception as to the extent of its influence. In his paper on "Gastrulation" ('06), he defines cephalogenesis and notogenesis thus: "The distinction

TEXT-FIG. 2.

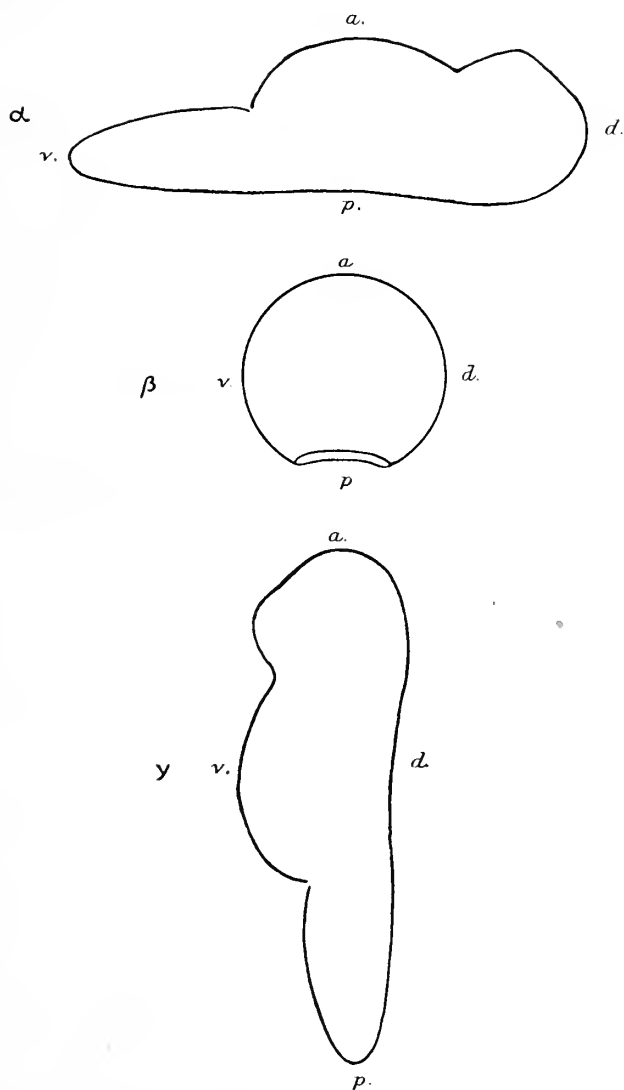


Diagram to show the difference in the conception of cephalogenesis and notogenesis on the one hand and protogenesis and deutero-genesis on the other. The middle figure, β , represents the gastræa or early cœlenterate stage, with blastopore on the lower surface. The upper figure, *a.*, represents the vertebrate according to Hubrecht, derived by elongation of the gastrula in the direction *vd*, producing an elongated actinian stomodæum, followed by concrescence of the walls of the stomodæum to form the notochord. The lower figure, γ , represents the vertebrate according to the author's conception by elongation of the gastrula in the direction *ap* by activity of cells forming the blastopore lip. Subsequently, the activity of the ventral part dies out, and the more dorsal part continues active and forms the tail.

here intended between 'kephali' and 'notos' is not identical with that between head and trunk (trunk segments having been ascertained to enter into the composition of the head), but that on one side should be ranged the very foremost portion of the head to which the ophthalmic [olfactory?] and optic nerves belong, whereas on the other we place the further subdivisions of the brain with their cephalic nerves, as also the basis of the skull with the remains of the notochord it contains, the visceral arches and the whole of the trunk."

I am inclined to think that a very considerable part of the gut, including the pharynx, and certainly the heart, are proto-

TEXT-FIG. 3.

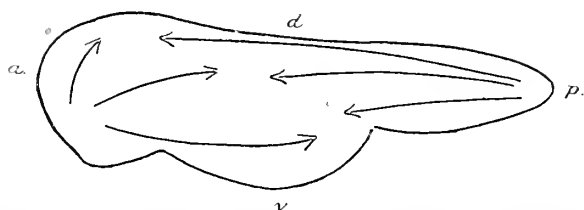


Diagram of a vertebrate to show approximately the parts due to protogenesis and deuterogenesis respectively. *a*, anterior; *d*, dorsal; *p*, posterior; *v*, ventral surfaces.

genetic. But the details of this form a subject for further experimental research.

Then in his paper of last autumn Hubrecht says (p. 43): "I think we may safely say that by the rapid extension backwards of the differentiation process, . . . the dorsal region of the trunk is laid down in outlines (hence the word notogenesis), whereas the derivatives of the ventral mesoblast find employment in the construction of the posterior and postero-ventral portion of the embryo."

The ventral mesoblast, by which Hubrecht means the mesoblast proliferated from the posterior or ventral lip of the blastopore, is, so far as the mesoblast is concerned, the evidence of the extent of the effect of deuterogenesis on the ventral

wall of the embryo, and as long as it is distinguishable from the protogenetic mesoblast (Hubrecht's protochordal plate and annular zone mesenchyme) its anterior margin forms a landmark between the protogenetic and deuterogenetic areas.

I have on a later page referred to a difficulty with reference to the notochord. But I will here ask Hubrecht two questions:

Is any part of the notochord formed—to use his own terminology—by kephalogenesis? That is to say, is there any notochord anterior to his supposed vermactinian stomodæum coalescence? If he answers No, then I will ask how he explains his earlier observation, e. g. on *Sorex*, where the protochordal plate is shown to give rise to the anterior portion of the notochord. If he answers Yes, then I will ask how can it be due to coalescence of the vermactinian stomodæum?

CHAPTER II.

So far as my own personal observations go I can support Hubrecht's contention as to the origin of mesoblast (mesenchyme) from hypoblast in front of the primitive streak, both as regards that in connection with the protochordal plate and that from the annular zone in the sheep and rabbit.

Hubrecht describes the peripheral mesenchyme-producing region in these words: "As an elongated ring-shaped zone of entoderm which is situated under and somewhat outside the border of the ectodermal shield and which, standing backwards from the protochordal plate both right and left, meets under the hinder part of the shield in the region where the mesoblast has acquired that median thickening which is known as the primitive streak, continued in the Primates into the connective stalk (Haftstiel)."

From my point of view this protochordal mesenchyme and annular zone mesenchyme which reaches round posterior and ventral to the primitive streak mesoblast is the mesenchyme of protogenetic origin, while the primitive streak

mesoblast, including Hubrecht's ventral mesoblast, indicates the extension of deuterogenetic mesoblast. This is approximately shown by the dotted line of the figures in my paper on the primitive streak of the rabbit, 1894, though the dotted line should have been considerably nearer the "embryo" (vide Assheton, 1898, p. 246, on sheep).

Hubrecht, on pages 35-37, disputes the view that there is any forward extension of material from the primitive streak area to form a *Kopffortsatz*, and holds that the protochordal wedge becomes lengthened, "not, however, by its sending out any 'Fortsatz,' but by its being, so to say, 'spun out' in consequence of the backward growth of the tissue that is going to be the notochord," with which description I am in sympathy, for it is the view put forward by me and illustrated by the diagrams on Plate 22 of my 1894 paper on the primitive streak.

At the same time I think this view must be slightly modified in accordance with the results obtained by actual experiment on the growth of the embryo in the chick (Assheton, 1896), which show that the "primitive streak" in the bird actually becomes converted into the embryo. It may seem a rather subtle difference, but really the elongated part of a primitive streak such as one sees in bird or rabbit is the stretched-out anterior part of the product of the deuterogenetic centre of activity rather than the anterior part of the deuterogenetic proliferative area itself, this product becoming subsequently differentiated directly into mesoblastic somites (which are always deuterogenetic), the deuterogenetic part of the neural plate, and of the notochord, etc.

Hubrecht then goes on to describe another growth centre, namely that which gives rise to the ventral mesoblast, which he says has preceded the formation of the protochordal wedge, and refers to four of his figures, 47-50, which, however, do not to my mind indicate any marked distinction either in space or time. Surely the so-called third centre of growth is nothing more than the equivalent of the ventral lip of the blastopore of *Amphioxus* and other *Anamnia*, and is the

ventral portion of the naturally circular deuterogenetic proliferation area.

This area is essentially circular in itself, but it assumes a different shape by reason of varying conditions and is a circular ring in Anamnia, which have blastopores like *Rana*, of an elongated ring- or bottle-shape (v. Sedgwick, 'Quart. Journ. Micr. Sci.,' vol. 33, p. 564) in Elasmobranchs, and streak-like in many mammals, e.g. rabbit, Carnivora-Tarsius and birds, truncate and almost disc-like in other mammals, e.g. *Mus*, *Cavia*, and reptiles. But in all it must have anterior, lateral and ventral margins from which proliferation of cell material takes place. I cannot conceive on what grounds Hubrecht can separate the anterior part (his protochordal wedge) and lateral wings from the ventral part. The figures he refers to do not support it, and besides, it is well known that the mesoblast sheet formed by deuterogenesis is a continuous sheet passing posteriorly completely round the primitive streak, broken only in front by the separation of the notochord.

The so-called extra-embryonic cœlom which develops so early in Tarsius and man is probably protogenetic cœlom, as it is well ventral to the posterior sheet of mesoblast (Hubrecht's ventral mesoblast), figs. 49, 50; and indeed, in the former figure, 49, it appears to be distinctly marked off from it. Moreover, the extra-embryonic cœlom develops long before any trace of the primitive streak, whether protochordal wedge or ventral mesoblast is present.

Hubrecht seems to have abandoned the position he took up in 1890 when writing on the embryology of *Sorex*. At that time he was sure that the middle region of the protochordal plate gave rise to the notochord:

"A most remarkable fact, to which I must now call attention, is this, that it is not in the posterior region of the epiblastic shield that the formation of the middle layer and its earliest representatives—notochord and lateral mesoblast plates—is first inaugurated. It is in the hypoblast that the first differentiation occurs, which ultimately leads to the

formation of the above-mentioned structures" ('Quart. Journ. Micr. Sci.,' vol. 31, p. 508). Again: "This patch of modified hypoblast-cells has at the beginning an oval shape, with the long axis perpendicular to that of the embryonic shield. Part of this patch will develop into the anterior portion of the notochord; for this reason I will call it the protochordal plate."

How does Hubrecht reconcile the formation of part of the notochord (what I should call the protogenetic portion of the notochord) from the gut lining with his theory of the origin of the notochord from the supposed fused Actinian stomodæum according to his present theory? (p. 169). Does Hubrecht abandon his earlier conclusions, or does he allow that some of the notochord is not formed from the stomodæal protochordal wedge?

In my humble opinion the Professor was on much sounder ground in 1890 than now, although, as related elsewhere, I cannot follow him in the idea of two gastrulation periods, an hypothesis which he has, as we have seen, abandoned since.

But surely the difficulties of cœnogenetic and palingenetic tissues, of mesoderms of various origin, of notochord of epiblastic or hypoblastic origin, are to a large extent smoothed away and the problem vastly simplified by an appeal to the way the things actually grow in the embryo as evidenced by experimental observation.

For we find that there is a part of the organism formed, so to speak, on the egg—in situ—roughly radially symmetrical, which is what I call the protogenetic area, and this alone represents a gastrula or cœlenterate stage.

To this subsequently is added tissue from a growing point (the deuterogenetic area of proliferation), which area itself can be called neither epiblast, hypoblast, nor mesoblast; but the material produced by active proliferation of this growing point becomes epiblast, hypoblast, or mesoblast, according to the nature of the layer with which it is in direct continuity.

The protochordal plate and the annular zone mesoblast,

with, of course, the hypoblast from which these parts are derived, together with the epiblast which overlies them, as well as everything ventral to the annular zone, constitute the protogenetic tissues, which are of more ancient origin than the primitive streak tissues, and everything this secondary area of proliferation gives rise to (deuterogenesis) are of a more recent origin, and include the protochordal wedge of Hubrecht (Kopffortsatz of others), the so-called ventral mesoblast and the lateral plates of mesoblast which are really one and the same thing.

I entirely agree with Hubrecht's comparison of these areas in question with corresponding ones in Amphibia, pp. 46-54, though I claim to be not included among those who were so "naturally biased" as to fail to see these points in Amphibia (vide 'Quart. Journ. Micr. Sci.,' vol. 37, Pl. 24, figs. 8-11, 13, 14).

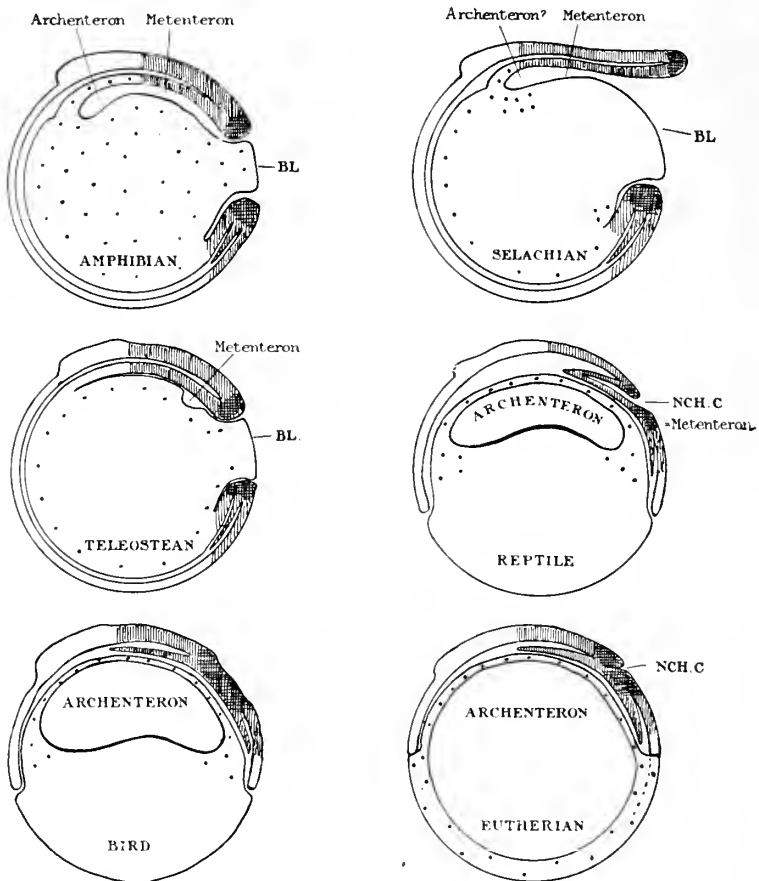
I have also indicated in the figure herewith reproduced from a paper in the 'Guy's Hospital Reports,' 1907, the respective regions formed by the protogenetic and deuterogenetic centres in various groups of vertebrates, though in these I have omitted details such as the annular zone of mesenchyme producing hypoblast, which is all included in the general white area marked with black dots, but the essential features are unmistakably indicated.

Hubrecht (p. 55) refers to birds and reptiles, and has no difficulty in finding evidence in them as in the Amphibia of the truth of the presence of two great embryonic growth centres in a vertebrate (protogenesis and deuterogenesis), and finds the same parts, protochordal plate and annular zone, protochordal wedge and ventral mesoblast.

I am inclined to doubt, from my own observations on the sparrow, whether the well-marked mass of cells which occurs in the sparrow at an early stage between hypoblast and epiblast, shown so clearly in Schauinsland's figures and called by Hubrecht "protochordal plate," is really the material from which any of the notochord is formed. If followed it is found to gradually take up a more and more forward position, and

may possibly form the extreme anterior part of the heart and pericardium, but in any case I am inclined to think it is

TEXT-FIG. 4.



Diagrams representing sagittal sections of vertebrates at corresponding ages to show the parts of the embryo derived from the primary growth centre (protogenesis), and that from the secondary growth centre or blastopore lips (deutero-genesis). The latter, the deutero-genetic tissues, are shaded. The dots represent nuclei within the yolk mass and endodermal tissues of protogenetic origin. (From 'Guy's Hospital Reports,' 1907.)

altogether anterior to the notochord, and is connected with the vascular system rather than that organ.

In reference to the relation between the protochordal plate and the secondary growth centre, Hubrecht (p. 58) writes: "The confluence between the earliest ectodermal downgrowth with the protochordal plate has up to now not been specially examined in reptiles. Still, one may conclude from the figures here given, which I copy from other authors, that it comes about in exactly the same way as we noticed it in *Tarsius* for Mammals, and in *Hypogeophis* for Amphibians."

Certainly the condition of *Hypogeophis* is more like the reptilian condition than is the condition such as occurs in the *Anura* or *Urodela*, in which there is from the first an opening from the exterior into the future archenteron, namely, a true blastopore.

Therefore, in comparing reptilian gut formations with others, we ought to compare it more closely with mammals and birds, and contrast it with Amphibians such as *Anura* and *Urodela*, and with fishes and cyclostomes.

Might it not be well to restrict the term "archenteron" to the part of the gut which is due solely to protogenetic influence, and call the part of the gut (including neurenteric canal) which is due to deuterogenetic influence by some such term as "metenteron" (vide diagram). If this is a true distinction, then we see that there is a very marked difference between the Amniotes on the one hand and all the other vertebrates.

In the Amniotes there is an archenteron formed by infiltration of fluid between an upper and anterior wall of cells, usually a thin membrane, and a lower or ventral mass of either cells or yolk mass, which, when formed, is not in open communication with the exterior—that is to say, there is no real blastopore. This cavity is, of course, that known as blastocyst cavity in mammals, subgerminal cavity in birds and reptiles. It is only at a later stage after the growth in length has started by the origin of the deuterogenetic centre that a passage is formed which varies very much in its degree of development in different types. Thus in birds it is only recognisable as a narrow and evanescent canal, the neurenteric

canal; in mammals it is usually not more developed, but in a few cases it is for a while recognisable as a distinct perforation or neurenteric canal, as, for instance, in the human embryo (Spee), the hedgehog (Hubrecht), *Ornithodelphia* (Wilson and Hill). But in the reptiles it is so evident as a passage leading at first into a blind pouch (but later communicating with the subgerminal cavity) that it has been mistaken for a true archenteron and true blastopore.

The true archenteron or subgerminal cavity is not so well marked in some (*Tropidonotus*) as it is in others (*Lacerta*, *Platydictylus*); but in all the "metenteron" is obvious, and subsequently a perforation occurs, and communication between the protogenetic cavity (the subgerminal cavity or archenteron) and the deuterogenetic cavity (neurenteric canal or metenteron) is established.

And I do not think this is a subtle distinction only, but one which is essential to the understanding of the true way in which the vertebrate embryo grows, and to my mind it tends towards simplification.

If it were not for the hypothetical vermactinian ancestor I see no reason why Hubrecht's and my own views should not absolutely coincide. For Hubrecht quite agrees with the view that this so-called invagination cavity of reptiles, this "cavity slit or porus," is not archenteron, and I believe that if he would himself perform operations on developing Amphibian, Avine, or Teleostean eggs, he would convince himself that the relations of the protogenetic and deuterogenetic centres are morphologically and physiologically as I have several times indicated them to be.

GASTRULATION IN THE ORNITHODELPHIA.

With reference to the description given by Wilson and Hill ('07) of the corresponding stages in *Ornithorhynchus*, I should like to make one suggestion.

Hubrecht accepts their interpretations, and regards the curious mass situated some distance in front of the primitive

streak, and called by Wilson and Hill the "primitive or archenteric knot," as the equivalent of the protochordal wedge of his nomenclature. Nevertheless I think Hubrecht is not very happy about it, as he says: "However, the data concerning the earliest appearance of this protochordal plate in *Ornithorhynchus* are too scanty than that I have ventured to mention it when in the preceding pages we discussed the protochordal plate. And it seems advisable on this point to await yet further researches on these rare mammals, of which it is so very difficult to obtain the required developmental stages."

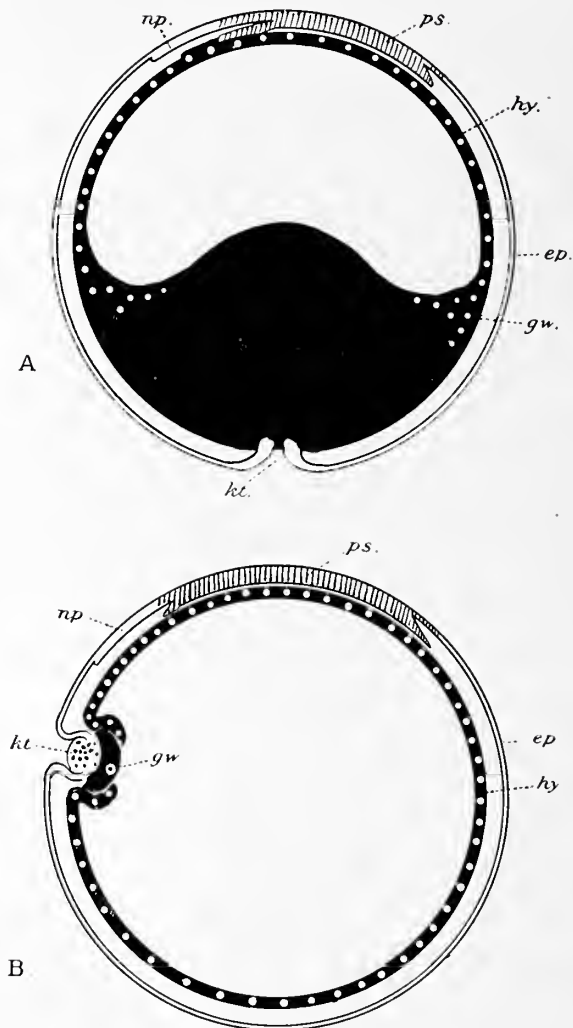
In spite of this no doubt admirable caution, I venture to suggest that the object called primitive knot by Wilson and Hill (*pr. K.*, text-fig. 7, Wilson and Hill) has nothing at all to do with either gastrulation or primitive streak, but is really the morphological vegetative pole of the egg.

I have tried to make my meaning clear by the accompanying diagram (text-fig. 5).

A. represents a meroblastic egg, such as that of a sparrow. In this the "liquefaction" of the yolk is seen very obviously to result in an accumulation of fluid between the blastoderm and the yolk mass, which forms a mass at the lower pole of the whole egg. The diagram is, however, not an exact representation. The epiblast, *ep.*, is shown to have nearly surrounded the yolk, a condition which is in reality not attained until a much later stage of development of the embryo itself than is indicated by the upper pole of the egg in my diagram, which represents a sagittal section of an early stage, perhaps eighteen hours or so of incubation.

Let us imagine, however, this growth of the epiblast, *ep.*, to take place very early (so that the yolk is completely enveloped by it) and the thickened edges to meet and fuse early. Let us imagine that the yolk is much reduced, and its place taken by fluid, so that the nucleated margins (*gw.*), the so-called germinal walls, have met and fused also; we should then have a condition not unlike the fig. B, except that in fig. B the part representing the coalesced blastoderm

TEXT-FIG. 5.



A represents a composition of two stages in the development of the egg of a sparrow, that is to say, the "liquefaction of the yolk" and the enclosure of the yolk by the epiblast are supposed to have taken place several hours earlier than is the case. B represents the blastocyst of *Ornithorhynchus* after Wilson and Hill's description. It is suggested that the "primitive knot" of those authors represents the final stage in the "liquefaction" of the meroblastic egg. *ep.*, epiblast; *gw.*, junction of hypoblast with yolk-mass—germinal wall of Balfour; *hy.*, hypoblast; *kt.*, "primitive knot" in B, and ventral pole of yolk-mass in A; *np.*, neural plate; *ps.*, primitive streak.

margins (epiblast and germinal walls) are eccentric. Now, is this a possible explanation of the primitive knot of Ornithorhynchus?

Fig. B represents a sagittal section of the monotreme egg, drawn from Wilson and Hill's description, except that the layers are shown too thick, and the primitive knot, *kt.*, is larger than it should be.

On this interpretation the section illustrated diagrammatically by Wilson and Hill, p. 51 of their paper, should be lettered thus: "*c.p.* cell plug" = fused edges of epiblast; "*ent.* yolk entoderm" = hypoblast; "*m.z.* marginal or cortical zone" = germinal wall or only remains of general yolk mass not yet liquefied; "*c.z.* central or more cellular zone" is probably also fused edges of epiblast, the edge more or less intermingled with the germinal wall yolk-cells.

The reason why it is not diametrically opposite the centre of the embryonal area is to be sought for chiefly in the greater expansion of the posterior arc or segment of the sphere. Such a condition is to be found in the rabbit's blastocyst, but in a very much less marked degree. If a blastocyst of a rabbit is taken out of the uterus at about seven days sixteen hours, i. e. just before it becomes attached by its lower pole to the walls of the uterus, it will be found to be of an oval form, with a smaller and acuter anterior and a larger and obtuser posterior end, and an area more or less ventral which will be devoid of hypoblast. The centre of this area is not diametrically opposite the centre of the developing embryonal area, but more towards the anterior end (vide Assheton, 1894, Pl. 17, fig. 47).

My suggestion is that the position of the primitive knot is due to a still more marked effect of a similar cause, the cause being the activity of the primitive streak or deuterogenetic centre, which allows of the more rapid expansion of the posterior segment of the blastocyst.

On this interpretation the primitive knot is of little importance, and the Ornithorhynchus egg appears as a condition intermediate between that of a reptilian and that of an avine

egg; reptilian because the neurenteric canal (metenteron) is so much more marked in the later stages than in any bird or even any other mammal, and avine because of the far greater rapidity with which the subgerminal cavity forms and swells up by infiltration of fluid and the great lengthening of the primitive streak, which is so characteristic of all birds and is so little marked in reptiles, though fairly well marked in some Eutherian mammals, e. g. rabbit, carnivora, ungulates.

My reasons for suggesting the above are that I cannot quite follow Wilson and Hill in their identification of the "embryonic region" of their fig. 5, Plate 2, with the structure in question, called "primitive knot," fig. 11, Plate 5, or of this primitive knot with the neurenteric canal or protochordal wedge development of the later stages, their archenteric knot of their text-fig. 10 on p. 73.

Very considerable gaps in their material occur between these three stages.

The earliest of the three stages, *a*, showing the primitive plate, is a blastocyst measuring 6×6.5 mm.

The middle stage, *Q*, was found in four eggs which measure 10 mm. \times 9.5 or 9 mm.

The last of the three, *P*, measures 17.5 mm.

Comparing the three stages with corresponding ones of a rabbit:

1st, *a* = 120 hours.

2nd, *Q* = 168 hours.

3rd, *P* = 188 hours.

As regards the first the comparison is difficult, because I cannot see that there is any real ground for considering that any layer present in the platypus is the homologue of the trophoblast, unless—which is not at all improbable—the mass of yolk and cells forming part of the so-called "primitive or archenteric knot" (= germinal wall?) is the mass of yolk-cells into which the epiblast in the Eutherian mammal's egg has sunk, and by which it becomes temporarily covered.

But the second stage, i. e. Specimen *Q*., fig. 7, is very much like the Eutherian mammalian embryo of the type which

resembles more closely the Avine embryo, such as a rabbit at 168–178 hours, except that the mesoblast, especially of the anterior region, is much more abundantly developed.

Wilson and Hill's figs. 16 and 15, Pl. 5, on this interpretation pass through what Hubrecht would call the protochordal plate, showing mesoblast or mesenchyme, which shows no sign of having been derived from primitive streak. There is not a hint of any proliferation of cells from the thickened epiblast above. In respect of the mesoblast or mesenchyme development the condition is far more Avine (cf. sparrow) than Eutherian.

So fig. 14 is perfectly typical of a transverse section through the front end of the primitive streak of bird or mammal at this early stage, i.e. when the primitive streak has attained its full length and before the counteracting effects of the primary and secondary growth centres have reached their highest development.

In fact there are only slight details which distinguish it from an embryonal area typical of those that develop upon a vesicle tense under hydrostatic pressure.

There is nothing at this stage to suggest any connection between this embryonal area and the curious mass of cells and yolk, *pr. K.* (Wilson and Hill's "primitive knot").

Why should not this embryonal area develop according to the plan upon which, say, the sparrow embryonal area develops in which a neurenteric canal is produced though not quite in so marked a manner (*vide* Schauinsland's figure, Hubrecht, fig. 110)?

In text-fig. 8 the next stage is illustrated, by which time, according to Wilson and Hill, the embryonal area of text-fig. 7 has expanded on the whole symmetrically so that the general outlines of mesoblast, thickened epiblast, and area opaca retain the same general relations to one another, the primitive streak, however, becoming slightly shorter, and occupying a relatively more posterior position.

This is exactly what occurs in similar types of embryonal areas in birds or mammals like rabbits. Compare, for instance,

Duval's 'Atlas,' figs. 161 and 182, and Assheton, 'Quart. Journ. Micr. Sci.,' vol. 37, Pl. 20, figs. 4A or 5 and 7 or 8.

Why is it not an expansion within the area itself in *Platypus* just as it is in the rabbit or bird?

What reason is there to suppose that there is in *Platypus* an extension forwards in some quite original manner so as to include a spot some distance anterior to it, except for the difficulty the authors felt in explaining this spot?

I would therefore submit to them the above suggestion as a defensible alternative explanation. And again, the archenteric knot is in no wise histologically like the part of other vertebrates to which on Wilson and Hill's hypothesis it is supposed to be homologous.

They had four eggs showing this spot, namely specimens D, DD (twins), Q and Y.

They give photographs of sections through Y and DD, a semi-diagrammatic figure in their previous paper of one (of the others?), and also a schematic mesial plane reconstruction of Y.

Now it must be obvious to everyone that the character of the dorsal lip of the supposed blastopore has nothing in common with the character of the dorsal lip of the blastopore of *Amphioxus*, or *Triton*, where it is concerned with gastrulation by invagination, or *Rana* or *Ceratodus* or *Scyllium*, where it is also concerned with gastrulation but less obviously with invagination, nor even with the anterior margin of the "mesodermsäckchen" in any reptile, bird or mammal (where it has nothing to do with gastrulation) either.

In all these it is a solid lip of more or less columnar actively dividing cells, making a compact thickened epithelium of very even and regular character, e.g. figs. 417, 418, 419, Hertwig's 'Handbuch,' vol. i.

In this *Platypus* "primitive knot" we see from fig. 2, Wilson and Hill, 1903, quite a different nature of epithelium. The so-called "dorsal lip" is an inactive layer of thin squamous cells passing into an uneven sac utterly unlike any other dorsal lip hitherto described.

Also the two structures are unlike in other ways. The primitive knot appears not very different from a yolk-laden reticulum with few nuclei, while the histological characters of what is alleged to be the same spot of the later stage present the crowding of nuclei usual to the front end of the primitive streak.

It is hardly likely that so fundamental a process as the formation of the neural plate—an organ whose formation in any other vertebrate is the earliest of all organs—should be represented by a squamous epithelial layer at a time when the primitive streak and mesoblast is fully established; especially unlikely is it when at this very time a plate of epiblast comparable in all respects, form, position, and time of origin, to a normal neural plate, already exists in its correct position, as shown by figs. 16 and 15 in Pl. 5, and text-fig. 7 of Wilson and Hill's 'Phil. Trans.' paper.

The next stage, called by Wilson and Hill post-gastrular, is represented by four eggs, *E*, *F*, *P*, and *PP*. The diameter of the first-named was 12.5 mm.

This stage is represented by their text-fig. 8.

In this it is supposed that the embryonic area has expanded in such a way as to include now the primitive knot.

In text-fig. 8 primitive knot, henceforth called by the authors the "archenteric knot," is shown as abutting upon the front end of the primitive streak. But the primitive streak itself has not increased in length, in fact it has slightly diminished.

In fig. 7 the archenteric knot is 22 mm. in front of the primitive streak.

In fig. 8 it touches it.

In text-fig. 7 the primitive streak is 39 mm. in length, in text-fig. 8 it measures 35 mm.

In text-fig. 8 the posterior end of the primitive streak is 8 mm. further off the posterior limit of the mesoderm than in text-fig. 7.

How can the junction of the archenteric knot and anterior end of primitive streak have been brought about? Has the

former ploughed its way back so as to join up with the anterior end of the primitive streak? This is incredible.

Has the primitive streak actually migrated forward to meet the knot?

Unless it has been accompanied by the mesoblast there is no evidence of a possible advance beyond 8 mm. and the distance to be traversed is 22 mm. (in the figures).

Has the primitive streak stretched or differentiated forwards?

No, it is actually shorter by 4 mm. than it was. Surely it is simpler to conclude that there has been no junction, but that the embryonic area has developed in an ordinary fashion like other mammalian embryonic areas, and that the so-called archenteric knot was really the remnant of an almost liquefied yolk-mass, and has now become so much further reduced as to be unrecognisable. And its position is, as before, well in advance of the anterior border of the embryonic area.

When we come to look at the photographs of the sections of the archenteric knot in the post-gastrula stage and compare them with the gastrula stage we notice that the histology is very different, for, instead of a mass of yolk with few small nuclei we have a quite different tissue, a compact cellular tissue crowded with nuclei.

To sum up, my suggestion is as follows:

(1) The primitive knot is the remains of the reticulum which contained the yolk mass.

(2) The "primitive knot," therefore, forms no part of the so-called embryonal area.

(3) Gastrulation occurs as in other Amniotes by infiltration of fluid forming a subgerminal cavity.

(4) The embryonic area of the authors, text-fig. 7, expands into that of text-fig. 8, as corresponding ones do in mammals like pig or rabbit, or in birds.

(5) The mass of mesoblast lying in front of the primitive streak of text-fig. 7 is the protochordal plate which differentiates into the anterior part of the vascular system, notochord (Wilson and Hill, archenteric plate), and mesoblast.

(6) The front end of the primitive streak becomes heaped up into a mass of tissue, the protochordal wedge, which, fusing with the tissues in front, adds, as the primitive streak recedes, deuterogenetic tissues to the notochord, nerve plate and other tissues affected by the growth in length.

(7) The boundary between the protogenetic part and deuterogenetic part of the notochord may be between the part of the notochord (archenteric plate) which is described by the authors as having a smooth under-surface and the part to which pieces of the broken-down "Mesodermsäckchen" are attached.

(8) A neurenteric canal is here formed as in some other mammals and birds, by the interaction of the two great growth centres, but on a much more marked scale which is exceedingly reptilian in its characters.

If we accept the author's nomenclature and regard the Mesodermsäckchen as archenteron, we are brought back to the old difficulty of being unable to account for the subgerminal cavity. If, once for all, we abandon all idea of a true blastopore in any Amniote, and regard the subgerminal cavity of birds, reptiles, and blastocyst cavity of mammals as homologous and all as archenteron, the result of protogenetic activity, and if we regard all subsequent pores which are invariably connected with growth in length as metenteric (to use the term suggested before) and deuterogenetic in origin, the matter seems to me to be greatly simplified, and is in complete accord with the results of experimental inquiry as to how things actually do grow in the living embryo.

CHAPTERS III AND IV.

PLACENTATION.

With the general trend of view in Chapter IV, which is admirable in all respects, few will wish to quarrel. It contains a clear account of the character of the trophoblastic changes in most of the mammalian orders. It is a matter of

satisfaction to myself to realise that the first paragraph on p. 101 confirms the conclusion to which I came a year or two ago, that we have, on the one hand, in the Ungulates and some other groups great lateral expansion of the trophoblast, leading to the type of placenta I called "plicate," and, on the other hand, cell proliferation of the trophoblast producing thickenings thereof leading to the type I called "cumulate."

But there is one point which Hubrecht alludes to only casually, namely, the part played by the uterine glands in the function of placentation. Maybe he does this purposely, because he proposes to deny the existence of a placenta to many forms of that type in which the glands play an all important part. Thus only forms like man, apes, insectivores, rodents, have placentas, while Equus, "Sus, Nycticebus, Galago, and others" have none!

"Attempts at systematic arrangements based on placental characters having never been very successful up to now, there is no objection to this somewhat radical change in our conception." But surely this makes confusion worse confounded! Therefore the sheep is a placental, the cow is a non-placental mammal!

We may not be able to agree over terms like "deciduous" and "non-deciduous," "voll placenta" and "halb placenta," "conjoined" and "apposite," "cumulate" and "plicate," but do not let us quarrel over whether a certain organ of nutrition or respiration formed in various animals from identically the same morphological units, appearing at the same time in all, having the same general function in all, disappearing at the same period in all, is to be called by different names according to the mode in which it takes in its nourishment.

Such a suggestion reminds one of Gideon's method of selecting men by the way in which they drank from the Spring of Harod. An excellent method of determining the particular merits of the men for certain purposes, but those individuals did not cease to be men who lapped like a dog.

I appeal to Professor Hnbrecht to let us retain the word "placenta" for all types of fœtal connection between mother and offspring for the purpose of nutrition, at any rate within the class Mammalia.

The size and shape of the uterus, the character of the submucosa, the nature of the uterine glands, are, of course, all features which have exercised profound effects upon the character of the attachment between mother and offspring, but I cannot see that they could be compared in morphological importance to the organ which alone makes them of any use at all. To say that the laterally expanded trophoblast and the distended allantois of the Ungulates' "placenta" is not an organ equivalent in all respects morphologically and physiologically with the more compact and confined mass of trophoblast and allantois of a hedgehog or rat, because the former obtains its nourishment largely from the glandular secretions, seems to me to be scarcely tenable.

No doubt the time is not yet ripe for using the placenta even as a broad means of classification, but Hubrecht's own work, especially that on *Tarsius*, has indicated that the placenta will be found eventually to have most important diagnostic value.

And I venture to predict that when that time does come the characters on which the main classification will rest will be characters of the allantois and trophoblast rather than maternal features, important though the latter may prove to be for the smaller subdivisions. As Hubrecht allows, the young blastocyst at any rate is parasitic in its relation to the mother; and is it not customary to classify parasites more by their own morphological and physiological features than by the effects which they have upon their hosts?

Although I did not suggest the terms "cumulate" and "plicate" as terms for a classification of mammalia on these lines, still for the present these terms seem to me to represent the essential character of two extreme forms of mammalian placenta. The characters of the two types may be tabulated thus :

Cumulate placenta.	Plicate placenta.
Great radial proliferation of trophoblast, leading to thickening of the membrane.	Great tangential proliferation of trophoblast leading to folding of the membrane.
Greater destruction of maternal tissue.	Lesser destruction of maternal tissue.
Much bleeding of mother.	Little bleeding of mother.
Lacunisation of trophoblast.	No lacunisation of trophoblast.
Secretion of uterine glands of less use or in some cases of no use to fœtus.	Secretion of uterine glands of prime importance.
Degeneration of uterine epithelium and glands severe.	Little or no degeneration of uterine epithelium and glands.
Embryo or embryos seldom fill the whole cavity of the uterus.	Embryo or embryos usually fill the whole cavity of the uterus.

The reason for the occurrence of the latter character being no doubt that fixation is brought about by imbedding or other intimate connection in the cumulate type, while fixation in the plicate type depends more upon the internal hydrostatic pressure of the blastocyst up against the uterus—much as a pneumatic tyre retains its position in the iron rim of a bicycle wheel.

All those are essential characters and are nearly all due to the nature of the trophoblast and allantois, i. e. of embryo rather than mother.

Modifications, especially within an order, may be due to the maternal influences. Thus, whether the embryo becomes wholly imbedded as in the rat, with typical cumulate character, or approaches the plicate type as in the rabbit (though the trophoblast undoubtedly shows its intrinsic cumulate character in the beginning) depends upon absence of the albumen layer in the former case allowing the embryo to be caught in the narrow chinks of the uterine wall and to become quickly parasitic, or presence of the albumen layer in the latter which compels the embryo to remain free for a much longer time before it can become parasitic.

Or as another instance may be mentioned the presence of certain areas—Hubrecht's trophospongia as in *Tupaja*, or the cotyledonary burrs of Ruminants which determine the spots

where either actual phagocytic attack may be made—Tupaja, Ovis, or only a mutual interdigitation may take place—Bos, Cervus, the intervening areas remaining free.

It has occurred to me to wonder whether the copious flow of uterine gland solution in, for instance, the intercotyledonary areas of Ruminants or the general surface of pigs, horses, may not obviate the necessity, so to speak, of the trophoblast eating into the maternal tissues by giving it an abundant supply of nutritive material.

I entirely agree with Hubrecht in regarding the Carnivora as a central group with respect to placentation from which either cumulate or plicate type could be evolved, or as connecting the two extreme types. Whether the carnivora, or the extreme cumulative or extreme plicate is the most primitive, it is very difficult to say. Hubrecht no doubt makes out a strong case for the cumulate type, except that it is based upon the non-sauropsidan origin of mammals, which seems to me untenable and unnecessary.

As strong a case might be made out by those who believe in the meroblastic egg and the sauropsidan origin of mammals for the other view. But, granted that the trophoblast is of yolk-cell or hypoblastic origin, I can see little difficulty in deriving either the cumulate or plicate type from the sauropsidan meroblastic egg.

AMNION.

One part of special interest is the discussion as to the origin of the amnion in general and in mammals in particular; and the question as to whether the present condition of the Eutherian development is to be derived from conditions which had their origin in a large meroblastic egg, or in a small holoblastic type, without the intercalation of a meroblastic stage. Hubrecht states his conclusions with great perspicacity in the second footnote to p. 79.

It seems to me that there is no necessity to give up the idea of mammals having been descended from animals with a

meroblastic egg, because certain things point to the probability of the type of Eutherian development such as we see in Insectivora, Cheiroptera and Primates (Anthropoidea), with cumulate placentation, being primitive as regards Entheria.

If we accept Hubrecht's hypothesis that the types just mentioned are primitive as regards the Entheria, we may nevertheless derive this from the large-yolked egg on the supposition that the trophoblast represents the reduced yolk-mass which has temporarily overflowed the inert epiblast, and yet retain the meroblastic type of egg for its pre-mammalian ancestry.

The only difficulty to which this leads one is that the amnion, such as we find it in Eutherian types like rabbit or sheep or pig, is not strictly homologous to the amnion of the Sauropsida. That an amnion so like as that of a sheep and that of a bird should be not absolutely homologous may seem to some improbable. Jenkinson ('00) objected to this view on a previous occasion. To whom I would answer thus—

In one sense all the amnions of the Amniota are homologous. There can have been no discontinuity in the presence of an amnion. The one kind cannot have ceased to exist until the other had come into existence. It is not a case of homoplasy only. But I submit that the amnion of the rabbit is not more homologous to the amnion of the sauropsidan than the horny teeth of *Ornithorhynchus* are homologous to the true teeth of the mammal or reptile, which they have supplanted.

A similar difficulty is with us already, within the small group of Eutherian mammals, and must be faced; for the amnion of the Eutherians, like the sheep, can hardly be strictly homologous to the amnion of *Cavia*, because whereas the former is formed partly of cells of the epiblast plate of the embryonal area and partly of trophoblast, the latter is formed wholly of cells of the embryonal epiblast.

In view of the fact of the curving of the epiblastic plate noticed in so many cases—*Sus*, *Tupaja*, *Talpa*, *Vespertilio*,

Cervus, Ovis, etc.—and particularly so in the case of the bat, to which Hubrecht draws especial attention, the idea that the formation of the amnion and the incurving of the epiblast of the embryonal area in Eutherian mammals may have had their origin in the overgrowth of the yolk by the epiblast after the manner of Sauropsidans as suggested by me (1908, p. 252; ref. Hubrecht, p. 23, line 19) seems to me still to have some value, although no doubt at the present time the retention of the curvature and the particular form of amnion formation in some mammals and not in others depends on various differences in present-time conditions.

This means that the rabbit, sheep, or dog type of amnion formation has been derived from a type like that seen in *Cavia*, whose ancestors had the sauropsidan mode of amnion formation.

The trace of such a history is perhaps to be seen in the blastocyst of *Capra* (Assheton, 1908).

ALLANTOIS.

I take it that the object of Hubrecht's suggestions as regards the Allantois is the removal of the difficulty of imagining how the Allantois could have acquired its respiratory or nutrient functions in the first place, and how it can have become attached to the somatopleure during early embryonic life in the second place, in other words, how to bridge over the gap between Anamnia and Amniota. Probably all agree that the Allantois as an organ of respiration, or respiration and nutrition, can only have come into being in connection with the presence of an amnion.

Hubrecht derives the amnion and trophoblast from an epiblastic larval envelope present before and when the ancestral Amniote became viviparous, and as yet having small, yolkless eggs. He discards the idea that the urinary bladder of the Amphibian could grow out like the allantois of an Amniote, and suggests that the allantois is the resulting effect of an original connection between the splanchnic meso-

blast of the gut and the larval envelope. I am not quite sure whether I understand his theory, but it seems that it must imply a connection having been made with the larval envelope by way of the lips¹ of the blastopore, and effected at an early stage of the growth in length, so that the allantois and its vessels represent a very much more anterior part of the gut than the urinary bladder of Amphibia, and that the hind gut arises as a dorsal diverticulum of the allantois. Or are we to believe that the hind gut of Amniotes is something new and that the anus of Amniotes is not homologous to the anus of Anamnia?

CHAPTER V.

The final chapter on the placenta is full of interest and less controversial than the rest of this comprehensive and remarkable treatise. Probably, however, most zoologists will be sorry that Hubrecht has not attempted to deal more thoroughly with the question of the value of the placenta as a guide to classification, though no one who has studied mammalian placentation will be surprised at his decision to postpone this most difficult subject. I feel, however, quite sure that Hubrecht must believe that the testimony is there to a large extent, and one will look forward to his exposition of it at some not distant date.

I entirely agree with Hubrecht's opinion that the diffuse placentation of the Lemurs is different from that of the true plicate forms of Ungulates, Cetacea, some Edentates, etc.

In my paper of 1906² I did, indeed, mention the Prosimia with much hesitation (vide Pl. 13) in connection with the plicate forms, but I felt then—and since having examined a specimen kindly sent me from the Zoological Gardens by Mr. F. E. Beddard, F.R.S., I have felt more strongly—that the condition of *Nycticebus* may possibly have been derived from that of

¹ I do not know, however, what in Hubrecht's opinion the relation between the larval envelope and the blastopore lips may have been.

² 'Phil. Trans. Roy. Soc. Lond.,' vol. clxviii.

a strictly cumulate type by way of such conditions as *Hylobates*, *Semnopithecus*, *Cercocebus*, by the gradual supersession of the glandular activity of the maternal uterus over the phagocytic activity of the foetal trophoblast, and the filling of the blood-spaces, into which the foetal villi originally hung, with uterine secretions instead of extravasated maternal blood.

The character of the foetal villi and maternal crypts are somewhat different in *Nycticebus* from those of plicate forms. In the plicate forms each villus fits into its own special crypt very exactly; the correspondence is, indeed, extremely intimate in some cases, e. g. the pig, where the processes of the trophoblast cells even penetrate between the cells of the uterine epithelium.

In my specimen of *Nycticebus* the foetal villi appear to hang in grape-like bunches into the mouths of much wider depressions, but this character may be artificially exaggerated in my specimen, as neither Hubrecht for *Nycticebus* nor Strahl for *Galago* indicate this character to the same extent.

Nevertheless, the difference between the true plicate form and *Nycticebus* is very well marked. In such plicate forms as *Equus*, *Bos*, *Tragulus*, *Orca*, *Halicore*, the foetal villi are filose, and fit into distinct crypts in the uterine mucosa, while in *Nycticebus* the foetal villi are more lobose and racemose, and can be described better as partially separated from one another by lamellate folds of the maternal mucosa, being elsewhere separated only by uterine gland secretion. The peculiar chorionic recesses described by Hubrecht ('94) are recesses where foetal villi project quite freely into a space filled presumably with uterine milk, as the recesses are open to the inter-foetal uterine space and certainly contain no projection of the uterine epithelium itself. Hubrecht says (p. 145): "*Nycticebus* has foetal investments which, in the latter half of the period of pregnancy, can, together with the enclosed foetus, be easily washed out of the maternal crypts, the trophoblastic villi not being in any way confluent with maternal tissue."

There is, however, no necessity to believe that the real plicate type has been derived from the extreme cumulate type, as is suggested for *Nycticebus*, nor even of the extreme cumulate from the extreme plicate. A middle view may be safer, namely, to regard the condition of the carnivora as that from which the two extremes have evolved, and that the Lemurs by some such process as that suggested above have given rise to a secondary condition from the more cumulate type, and simulate roughly the plicate type. The earliest stages of a Lemur have not yet been described. If there is anything in the suggestion above, the earliest formed trophoblast of the Lemurs like *Nycticebus* ought to show "cumulations."

I am entirely at one with the Professor when he recalls the great value of his discovery of the nature of the placenta of *Tarsius*, and not only the placenta, but the character of the blastocyst, the coelom, mesoderm, and allantois, which so closely resemble those of man that they simply cannot be ignored if comparative anatomy is of any value at all as evidence of racial affinity. But I think it is not necessary to take so strong a view as Hubrecht does of the difference between *Tarsius* and the other Lemurs in these respects, and I have indicated a way in which the Lemurine may have been derived from the Tarsian condition. The condition seems to me to emphasise the affinity between Lemurs and Monkeys rather than to necessitate the complete separation of *Tarsius* from the Lemurs. So far as it goes the comparison supports the view of Dr. Elliot Smith expressed in his letter in 'Nature,' vol. lxxx, No. 2054. It is, however, a condition quite sufficient to justify a grouping of the Lemurs into those with the pseudo-plicate "Lemurine" placenta and free allantois on the one hand and *Tarsius* with the cumulate placenta and early allantoic attachment on the other hand, the latter group being closer to the Monkeys than the former, as, indeed, has been shown by other characters.

CHAPTER VI.

Lastly, Hubrecht wishes to revise the classification of vertebrates in the light of this supposed homology of all outer trophic or protective layers within the phylum.

He says (p. 81): "And it then, of course, strikes us, supposing all these different outer covering layers during early larval life to be the remnants of an early larval envelope, of which we find no trace in *Amphioxus*, the cyclostomes, and the Elasmobranchs, that the deep significance hitherto attached to the foetal membranes as a means of sub-dividing the Vertebrates into the primary groups of Amniota and Anamnia runs great risk of losing much of its significance."

So Hubrecht, leaving out of consideration the Hemichordata and Urochordata, divides the higher chordates into four "super-classes," namely Cephalochordata (for *Amphioxus*), Cyclostomata (for the Marsipobranchs), Chondrophora (for the Elasmobranchs), and Osteophora (for the Amphibians, Dipnoans, Ganoids, Teleosteans, Reptiles, Birds, and Mammals).

Seeing that the bond of union for these last seven groups is the supposed presence of a larval envelope, one wonders that Hubrecht has not coined a word suitable to this phenomenon instead of falling back upon the presence and absence of bone. Does the neglect suggest a lack of faith?

On p. 17 Hubrecht writes: "A tendency to exchange the radial for a bilateral symmetry and to separate the coelom from the enteron must at one time have characterised certain coelenterate ancestral forms, as has already been advocated by Sedgwick ('84) and by myself ('05) on earlier occasions. It is not straining the imagination to assume that in this line of descent closely-related forms may have developed, some with, others without a larval envelope, temporarily ensheathing the cellular elements that will build up the embryo itself, and thus foreshadowing the separation among their later vertebrate descendants of such with and such others without a trophoblast."

I think it is clear from this paragraph that Hubrecht suggests that the separation into the great groups Osteophora with larval envelope on the one hand, and Chondrophora, Cyclostomata, and Cephalochordata without larval envelope on the other hand, dates back to cœlenterate days, or at any rate to his vermactinian ancestral phase.

How, then, are we to regard such similarities as the pineal eye of *Sphenodon* and the pineal eye of *Geotria*; or the first ten pairs of cranial nerves and their distribution in an Amniote with the ten pairs of cranial nerves of *Scyllium*; or, in fact, any of the hitherto supposed homologous parts which are to be regarded as comparatively speaking recent acquisitions within the phylum Chordata? Are we not forced to ask ourselves, Which of three alternatives are we to accept henceforth?

(1) That the anatomical resemblances such as those referred to above are not true homologies, but are accidental resemblances.

(2) That the cœlenterate ancestor "with a tendency to become bilaterally symmetrical," but not at that time having any organs at all visibly like the brain, sense organs, kidneys, skeleton, etc., of the Lamprey, Elasmobranch or Amniote, nevertheless was so constituted internally that its descendants, although separating along different lines of descent, as shown by the presence and absence of "trophoblast," attained closely similar results in spite of the divers effects that natural selection is known to have had in other divergent lines, the Ascidians and Enteropneusts.

(3) That Hubrecht's attempt to homologise all such superficial layers as the trophoblast of *Eutheria*, "teloderm" of *Sauropsida*, the *Deckschicht* of *Amphibia*, of *Teleostomes*, etc., fails.

Nor does Hubrecht seem to me to meet the objections urged by van Beneden, Gadow and others, namely, the many characters which unite the three groups forming the Amniota and marking them off from the rest of the Vertebrates. I may here mention a few and mostly embryological points

which are probably of prime importance (though some of these are already given in other authors' lists, e. g. Gadow, H., 'Zeits. f. Morph. u. Phys.,' Bd. 4).

Amniota.	Anamniota.
Amnion.	No amnion.
Allantois as respiratory organ.	No respiratory allantois.
Umbilical vesicle or yolk-sac posterior to and quite separate from liver.	Yolk-sac either anterior or in connection with liver.
No true blastopore. If any opening occurs to which this term has been applied it never occurs until after the first formation of the future gut cavity, i.e. it is a neurenteric canal.	Blastopore; always correlated with the first formation of the gut cavity. (Gymnophiona may be intermediate.)
Spiracle always present and open as long as any other cleft.	Spiracle often rudimentary or absent.
Peculiar epidermic skeleton of homologous parts (scales, feathers, hairs).	Nothing comparable.
Vertebral column gastrocentrous form of Arcocentrous type.	Vertebral column notocentrous and pseudocentrous forms of Arcocentrous type.
Ribs well developed and reaching sternum (except specialised types, e.g. Chelonia, Ophidia).	No ribs, or if present they do not reach the sternum.
Hypoglossal comes off within the skull.	Hypoglossal always outside the skull.
Neck.	No neck.
Metanephros in connection with a special outgrowth from the mesonephric duct to form the ureter.	No metanephros nor special outgrowth from the mesonephric duct.
Mesonephric duct never conveys urine in adult.	Mesonephric duct itself is ureter.
Pronephros of embryo rudimentary.	Pronephros of embryo well developed.

The only other point to which I will allude is Hubrecht's question, "Whether many of our Dipnoi, Ganoids, and Teleosts may not have had terrestrial ancestors," p. 153.

Naturally, I feel proud that I should have been bold enough to make a similar suggestion for the Teleosts in

my *Gymnarchus* paper in the 'Budgett Memorial Volume'; and although it is doubtless a "wild and hypothetical speculation," yet it seems to me to be an idea which deserves consideration by both embryologists and palæontologists. It is curious, however, that the reasons which led me to the idea are quite different from those which brought Professor Hubrecht to the same conclusion ('Budgett Mem. Vol.,' p. 407).

And although I was well aware of, and alluded to, the close resemblance between the trophoblast of Eutherian mammals and the Deckschicht of Teleosteans ("Report of Eggs and Larvæ from the Gambia River," 'Budgett Mem. Vol.,' pp. 434-6), it never struck me to regard this as evidence of affinity between the groups; and I cannot yet get over the difficulties of regarding these and the other outer layers discussed in the preceding pages as homologous, for the difficulties appear to me insurmountable.

In conclusion, may I again express my appreciation and admiration of the great work upon which I have ventured to comment. And if, in my excursus, I have settled more often and dwelt longer upon points where I have found myself in opposition to the Professor, it has been in the belief that such disputations tend towards the elucidation of the truth, and not because I am in any way insensible of the magnitude of the work, nor because I am not to a large extent in accord with the conclusions based upon it; for no one can realise more clearly than I do how much of the interest, which we who have the more recently been drawn to this field of research in mammalian embryology feel in the subject, is due to the brilliant researches and writings of Hubrecht.

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The Formation of the Layers in *Amphioxus* and its Bearing on the Interpretation of the Early Ontogenetic Processes in Other Vertebrates.

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With Plates 18, 19, 20, and 21, and 10 Text-figures.

THE readers of the Quarterly Journal of Microscopical Science have been treated in a recent number to a brilliant essay on the early ontogenetic stages of vertebrates from the well-known pen of Professor Hubrecht (18), who has dealt with the subject from the point of view of a specialist in mammalian embryology, and may be said to have envisaged the development of vertebrates through mammalian spectacles.

To every question there are at least two points of view, and I desire in what follows to lay before the readers of this journal another way of looking at the early development of Vertebrata, taking as my starting-point, not the highest members of the group, as Professor Hubrecht has done, but the lowest form which all zoologists are agreed in including within the phylum Vertebrata, viz. *Amphioxus*. Which of the two points of view will ultimately gain the greatest amount of support will depend, I am convinced, upon which offers the simplest and most natural phylogenetic explanation of the facts to be interpreted.

The first part of this paper will therefore consist of a re-description of the formation of the layers in *Amphioxus*. I say a re-description, because eleven years ago I gave a

description of the early stages of the development of this animal, based on hundreds of series of sections through well-preserved material (26). The conclusions arrived at in that paper, however, have been combated by several workers since, and as the figures illustrating it were hastily executed and somewhat schematic, owing to my impending departure from England to take up work at McGill, some authors have taken for granted that the material was badly preserved and that my observations were therefore of little value. Such a conclusion is entirely unwarranted, as the figures illustrating the present paper will, I hope, convince every unprejudiced observer. I have endeavoured, so far as my powers of draughtsmanship extend, to depict the cellular structure in minute detail, and I find, on going over the subject again after eleven years' interval, that I must re-affirm all the statements I made in 1898 with a few trifling exceptions in minor points.

PART I.

THE FORMATION OF LAYERS IN AMPHIOXUS.

Before, however, proceeding to the description of the development of *Amphioxus*, it is perhaps desirable to give a brief review of former work on the subject so that the questions at issue may be clearly grasped. Kowalevsky was the first who published a paper (21) on the development of *Amphioxus*, which he followed up some years later by a second paper on the same subject (22). In his first paper he describes the egg as dividing into blastomeres of nearly equal size, forming a hollow blastula. This blastula, according to him, is converted into a gastrula by a flattening of one pole, followed by an invagination of the flattened area proceeding equally at every point of its circumference, so that the blastopore is left as a symmetrically situated posterior pore. In his second paper Kowalevsky describes the formation of "folds" of the inner layer (hypoblast) situated dorso-laterally. From the context it is clear that by "folds" he means pouch-like outgrowths. He makes the significant

statement that the cavities of the first pair of these "folds" remain for a long time widely open to the gut, whilst the cavities of the others have very narrow communications with the gut and are soon cut off entirely from it. Further, he states that whilst the first pair of folds become converted into hollow epithelial vesicles, the cavities of the other pairs become quite obliterated. He was not able to trace these folds into the formation of the body-cavity of the adult, but he believed that a genetic connection existed between the two sets of organs.

After an interval of some years Kowalevsky was followed by Hatschek, who, in a brilliant paper, gave the account of the development of *Amphioxus* which has been incorporated in all the text-books of zoology (15). Hatschek pointed out that in the formation of the blastula the blastomeres at one pole are larger than at the other, and that it is the area consisting of the larger blastomeres which is invaginated when the gastrula is formed. He observed further that when gastrulation is complete the blastopore was situated at the posterior end of the dorsal surface. He drew the conclusion that in the early stages of gastrulation, when there is a wide-open blastopore, this is directed towards the future dorsal surface of the animal, and that it becomes reduced in size by the meeting of its edges, a process which he supposed to take place from the front backwards. Hatschek never asserted that he had seen this union or "concrecence" of the lateral edges of the blastopore; and it is hard to see why, if such a process really took place, some trace of it, in the form of a seam or raphe, should not be observed. Once the gastrulation has been completed the processes of formation of the notochord and of the mesoderm are begun. These processes, according to Hatschek, consist in the production of three longitudinal folds of the dorsal wall of the gut, of which the median forms the notochord and the lateral the mesoderm. These "folds" are genuine longitudinal hollow ridges, not "folds" in the sense in which the word is employed by Kowalevsky. It is important to note this because many who

have referred to or transcribed Hatschek's work appear to imagine that he described the mesoderm as originating as a series of pairs of independent pouches, which is not the case. According to Hatschek the lateral folds, whilst still in open communication with the gut, are divided by constrictions into four or five "somites" on each side, of which, however, only the first pair are completely separated from the rest, the others constituting a single moniliform fold on each side. A little later the front part of this moniliform fold becomes completely separated from the gut, and its constituent somites then become completely separated from one another, the last only retaining its communication with the gut. As new somites are formed the animal grows in length and the primary fold is prolonged backwards; from this fold these somites are cut off, and only the last pair at any moment is in open communication with the gut. The first pair of somites, however, which were early separated from the rest, long retain their communications with the gut. Each of the somites undergoes segmentation into a dorsal portion which constitutes the myotome, and a ventral portion, which fuses with its predecessor and successor to form the splanchnocœle, the right and left splanchnocœles fusing to form the general abdominal cavity of the adult. From the most anterior part of the gut two diverticula grow out which become cut off from it; these are termed by Hatschek "head-cavities"; of these the left becomes a small thick-walled vesicle, which subsequently acquires an opening to the exterior, whilst the right becomes a large thin-walled vesicle which forms the cavity of the snout in the young *Amphioxus*. It is evident that most of Hatschek's observations are to be regarded as extensions of Kowalevsky's results, due to his more perfect method of technique. The only point in which they radically differ is in the position they assign to the blastopore, and in this matter Hatschek frankly acknowledged that his conclusions were far from certain.

So clear and simple an account as that given by Hatschek remained for a long time in favour, but in 1894 it was attacked

by Basilius Lwoff (25). According to this observer the process of gastrulation consists of two parts; during the first stage there is invaginated the genuine large-celled endoderm, consisting of the larger blastomeres situated at the lower pole of the blastula; from this the epithelium of the gut is formed. During the second stage of gastrulation the small-celled ectoderm is invaginated round the dorsal lip of the blastopore, and forms the dorsal wall of the "archenteron" or gut of the completed gastrula. This ectodermal plate, however, is completely used up in the formation of the notochord and of the mesodermal folds; the edges of the endodermal portion of the gut meet beneath it, and so complete the definitive gut. The cavities of these folds stand in no relation to the future body-cavity of the adult, for they are produced by the pressure on the dorsal wall of the gut of the in-sinking nerve-plate, which is commencing to be invaginated, and their cavities subsequently disappear entirely, whilst the cœlom consists of spaces which arise subsequently as splits in solid masses of cells. It follows that mesoderm and notochord are of ectodermal origin, and that *Amphioxus* is in no sense an "enterocœlous" animal, nor are the processes of the formation of its layers capable of being analysed into simple processes of folding in the wall of an epithelial sac. Lwoff further denies that the blastopore closes in a slit-like manner from before backwards.

Lwoff's observations seemed to fit into what had been observed in the development of the higher Vertebrata. He emphasises this point, and ingenuously confesses that he would not have felt certain about his account of the development of *Amphioxus* if he had not found it confirmed by his observations on the higher Vertebrata. An account, however, so radically distinct from that given by Hatschek needed confirmation, and accordingly in the year 1898 four papers appeared on the subject by Sobotta (32), Samassa (30), Klaatsch (20), and myself (25). Sobotta deals only with the gastrulation. He denies Lwoff's assertion of a difference in histological character between the cells forming the dorsal wall of the archenteron and those constituting its ventral wall.

Lwoff had based his conclusion that ectoderm cells are inflected round the dorsal lip of the blastopore on his observation of numerous mitoses there; he asserted that they were wanting elsewhere. On this point Sobotta joins issue with him, and points out that mitoses occur frequently in all parts of the archenteric wall. Sobotta had not been able to accurately determine the orientation of his embryos, but Samassa, who wrote in the same year (29), using the method of double embedding in celloidin and paraffin, was able to determine this. Samassa's results in general confirm Sobotta's, but they add several most important points. He points out that mitoses take place at regular intervals in both endoderm and ectoderm, so that it is quite possible in one embryo to find no mitosis at all, and in another of nearly the same size to find numerous mitoses. Samassa further asserts that the blastopore closes by the synchronous approach of all its sides, and he rejects utterly the idea that it closes in a longitudinal seam. With regard to the formation of the mesoderm he confirms Hatschek's results; he points out that the longitudinal grooves which give rise to the mesoderm make their appearance before the nerve-plate becomes invaginated, and that therefore Lwoff's explanation of their formation is groundless. Klaatsch's paper on the gastrulation (20) contains several admirable new points. He remarks that the abundance of mitoses found by Lwoff in the neighbourhood of the dorsal lip of the blastopore does not prove an inflection of cells round this lip, but rather proves that here is to be found a growing point from which new cells are added to both ectoderm and endoderm. He denies the possibility of an inflection of cells round the dorsal lip, because of the difference in histological character between the ectoderm and the cells forming the dorsal wall of the archenteron. With regard, however, to the ventral lip, he infers that here there is a genuine inflection of cells, because he found outside this lip large rounded cells, which in later gastrulæ were replaced by small-celled ectoderm. My own paper (26) was published in ignorance of the results of the three workers whose observa-

tions I have just summarised. In it I denied that there was any histological difference between the cells forming the dorsal and those forming the ventral wall of the archenteron. I denied, also, that there was any inflection of ectodermal cells round the dorsal lip of the blastopore, but I asserted that the gastrulation was due, in the first instance, to a rapid increase of endoderm cells in this neighbourhood, which produced a lateral strain on the endoderm to which it yielded by invagination, that the blastopore was directed posteriorly, not dorsally, and that the closing of the blastopore, or, to speak more correctly, its reduction to a small pore, was due to the appearance of a new centre of rapid growth in the ventral lip of the blastopore. I asserted further that the first pair of somites were independent formations from the gut-wall, which I compared to the collar-cavities of *Balanoglossus*, and that the other somites owed their origin to the segmentation of a hinder pair of outgrowths from the gut-wall, which I compared to the trunk-cavities of *Balanoglossus*, and which grew in length *pari passû* with the growth of the animal, and that only the hindermost as yet unsegmented portions of these grooves retained openings into the gut. I further asserted that whilst the other somites divided into myotomes above and ventral portions below, which fused to form a continuous splanchnoceles, this was not true of the first pair (the collar-cavities). In these the dorsal myotomic portion remained in open communication with the lower thin-walled portion of the somite. This latter did not fuse with its successors to aid in forming the splanchnoceles, but on the contrary extended posteriorly outside the splanchnoceles forming the basis of the future atrial fold. In a subsequent paper (27) I corrected a hasty and ill-founded statement made in the first paper, that in the spherical blastula the blastomeres were all equal in size, and I showed that the backward extensions of the atrial cavity became solid, and apparently furnished the material for the transverse muscle of the atrial floor.

In the same year that this second paper was published, a

paper appeared by Morgan and Hazen (28), illustrated by beautiful figures. These authors support Lwoff's statement that there is a histological difference between the cells forming the dorsal and those forming the ventral wall of the archenteron. Their account of the matter is, however, very confusing. In one place they speak of "yolkless" cells being inflected at the dorsal lip of the blastopore; in other places, however, they represent the difference between the roof and floor of the archenteron as merely a difference in the intensity with which stain is taken up, and further assert that this difference cannot be seen in specimens preserved by corrosive sublimate. Their figures certainly show an immense difference between the ectoderm cells and those forming the roof of the archenteron, whilst the difference between the floor and the roof of the archenteron on which they lay such stress is only indicated by the colour of the yolk-granules. Morgan and Hazen do not accept Lwoff's view that the cells forming the roof of the archenteron are ectoderm, and they point out that mitoses in the dorsal lip of the blastopore are no proof of a migration of cells round the lip, and that, as matter of fact, mitoses are by no means confined to this lip but occur everywhere in both ectoderm and endoderm. They assert that in my earlier paper I had confused the dorsal and ventral lips of the blastopore, and they find a gradual passage from ectoderm to endoderm cells at the dorsal lip. After considering all the views which had up till then been put forward as to the manner in which the blastopore is closed, they consider the most probable account of the matter to be that the blastopore is directed dorsally, and is closed by the advance over it of its anterior lip. No further paper on the development of *Amphioxus* appeared till 1906, when Cerfontaine published an exhaustive paper on the subject, which, largely because it was illustrated by many beautiful figures, has been accepted as decisive by many zoologists (9). Cerfontaine is bitterly hostile to the conclusions reached in my work, and it is principally in order to see how far his criticisms are justified that I have undertaken

a re-examination of the whole subject, the results of which are presented in this paper. He gives an elaborate account of the segmentation, confirming Hatschek's account, and noting the presence of two circles of larger blastomeres near the vegetative pole. When the blastula becomes flattened, he finds that the invagination commences asymmetrically near what afterwards becomes the dorsal or anterior lip of the blastopore. In this he entirely confirms my work, without apparently being aware of it. But he maintains that small-celled ectoderm is inflected round the dorsal lip and forms the roof of the archenteron, thus re-affirming Lwoff's assertion. He arrives at this conclusion on two grounds: first, because of the existence of numerous mitoses near this lip (thus ignoring the criticisms of Samassa, of Morgan and Hazen, and of Klaatsch), and secondly, because of a histological difference between the cells forming the roof and those forming the floor of the archenteron. When we probe his figures for evidence of this difference, we find that it consists in the presence in the cells forming the floor of a few larger and more deeply staining granules of yolk than are found in the cells forming the roof of the archenteron! There is, however, a profound difference between these latter and the indubitable ectoderm. Cerfontaine also re-affirms Hatschek's surmise that the blastopore narrows in a seam-like fashion from in front backwards; but he no more than Hatschek has observed such a seam, and the reasoning which leads him to this conclusion is certainly recondite. In the process of the narrowing of a wide blastopore the lateral lips must come together; now he shows by measurement that during this process the dorsal and anterior lip moves backward, in other words, that the embryo grows longer. Hence he concludes that this growth of the dorsal lip must be due to the same process which narrowed the transverse diameter of the blastopore! Cerfontaine affirms in accord with Klaatsch that ectoderm is also invaginated at the ventral lip of the blastopore, basing his conclusion on the existence of mitoses here during the later stages of gastrulation. Verily Cerfontaine's

conclusions are like a pyramid standing on its apex! In spite of his hostility to me his figures really support my observations when relieved of the forced interpretation, which his espousal of Lwoff has led him to put on them. With regard to the formation of the mesoderm Cerfontaine has little new to contribute. He dismisses my observations with the remark that he has never seen anything like them in his preparations and that my material was badly preserved—a statement he thinks it necessary to reiterate three times in his paper, and which is absolutely untrue, and especially uncalled for in this case, as Cerfontaine's figures of the formation of the mesoderm are as schematic as those which I published in my first paper. Cerfontaine, however, confirms me against Lwoff in asserting that *Amphioxus* is an "enterocœlous" animal so far as the cavities of the first pair of somites are concerned. Legros, who had previously published a short paper (23) on the larvæ of *Amphioxus*, in which he had maintained that the epithelial vesicle, identified by Hatschek and myself with the left head cavity, was an ectodermal invagination, and that the club-shaped gland had no external opening, followed it up in 1907 (24) with a paper on the formation of the layers in this animal. Legros's work differs from that of all his predecessors in that it is based, not on normal, but on abnormal development. By fertilising ova with stale sperm and similar means he obtained some curious pathological embryos. In one of these there was a wide posteriorly directed blastopore, but the mesodermal grooves had been already formed and from these, two pairs of somites had already been separated off. There was also in existence a horizontal ridge on each side of the archenteron, tending to divide it into an upper section to which the ectodermal grooves belonged, and a lower one which is regarded by Legros as consisting of true endoderm. When the series of sections was followed backwards the horizontal ridges united and shut off the lower section of the archenteron, which ended in a *cul-de-sac*, from the upper section, which opened by the blastopore. In

another embryo which he describes development is much more advanced, and quite a number of somites have been formed. Nevertheless, not only is there a wide blastopore found at the posterior end of the animal, but also the same ventral diverticulum of the archenteron, and there is further evidence to be found that the front part of the blastopore has healed by "conrescence." Legros regards his observations as proving that Lwoff and Cerfontaine are right in supposing that the roof of the archenteron is ectodermic; and the horizontal ridges he regards as conveniently marking the boundaries of the ectodermal and endodermal portions of this sac; and he naturally regards his older embryo as proving that the blastopore closes by conrescence. It must, however, be remembered that in normal development the blastopore is reduced to its normal dimensions before any trace of mesodermal grooves or somites is formed, and the processes by which a pathological embryo adjusts itself to the normal condition of affairs are likely to be widely different from what occurs in the normal course of development. This is obvious even from Legros's figures; for if conrescence took place normally the tissue destined to form the notochord would be formed by the meeting in the middle line of two symmetrical halves; now, in Legros's later embryo the notochord is seen to belong entirely to one lip of the two which meet in the middle line. Further, in a normal *Amphioxus* so soon as the mesodermal grooves have been formed a great growth in length takes place largely localised in the hinder part of the embryo; now, if through any reason this growth was unequal we should at once get Legros's type of embryo without having any light thrown on what took place in normal embryos. Legros's abnormalities are, therefore, probably not due to disturbances in the processes of formation of the layers, but to disturbances in the process of growth in length which supervenes very early in *Amphioxus*. Legros agrees with Cerfontaine that *Amphioxus* is truly an enterocœlous animal, for he finds that the first pair of somites long retain their open connection with the

gut, and he prudently withdraws his earlier statement that the left head cavity was of ectodermal origin.

Reviewing the observations which have so far been summarised, we see that two questions stand out as pre-eminently calling for decision : first, is the roof of the archenteron, and consequently the mesoderm and notochord which arise from it, ectodermal or endodermal? And secondly, does the blastopore close by a seam-like concrescence proceeding from in front backwards or not? A third question to be resolved is whether the comparison which I instituted in 1898 between *Amphioxus* and *Balanoglossus*, so far as the formation of the mesoderm is concerned, can be sustained.

MATERIAL AND METHODS.

The material at my disposal consisted of a profusion of eggs and embryos covering all stages from the spherical blastula up to the period when the mouth, club-shaped gland, and one gill-slit have been formed. There were also a number of older larvæ, but a considerable gap intervened between them and the larvæ with one gill-slit, and in this paper I consider only the material which formed a series without any gaps. Next, as to preservation, the material was preserved partly in corrosive sublimate and acetic acid, partly in Kleinenberg's picrosulphuric acid, partly in Fleming's fluid, and partly in osmic acid. I had material of all stages preserved in corrosive sublimate and in osmic acid, and in the accuracy and fineness with which histological detail is preserved there is no other reagent which will stand comparison with osmic acid. This is, of course, a conclusion that is becoming more and more widely accepted, and some histologists go so far as to maintain that osmic acid is the only reagent which retains in dead protoplasm anything like the organisation it possessed in life. When, however, yolk is extremely abundant osmic acid makes the material so brittle that section cutting is almost impracticable; and for this reason, in dealing with the stages of gastrulation I have used material preserved in

picrosulphuric acid and in corrosive sublimate and acetic acid, but for all later stages osmic acid material alone has been used.

Samassa and I simultaneously employed the method of double-embedding in celloidin and paraffin, which, indeed, is the only method of dealing effectively with such minute and delicate embryos. As modified by me the procedure was as follows: The celloidin containing the embryos after being congealed in chloroform, was transferred to cedar oil. In this oil it became as clear as glass, so that the embedded embryo could be examined under the microscope and its orientation determined. An appropriately shaped piece of celloidin was then cut out and embedded in paraffin. The sections were stained on the slide. My conclusions so far as the gastrulation is concerned are based only on sagittal sections, the orientation of which was determined as I have described, but the accuracy of this orientation was tested by counting the sections in the series and observing whether the extreme ones at either side were alike in form. This is possible only where the direction of the sections is accurately sagittal. The median section in each series was then figured, and all the figures are drawn with about the same magnification, viz. 900-1000 diameters.

THE GASTRULATION.

We shall commence our study of the development of *Amphioxus* with the stage of the flattened blastula, for in this stage the axes of symmetry can for the first time be determined. A median sagittal section through an embryo of this stage is represented in Pl. I, fig. 1. The outline is hemispherical, and for reasons which will presently emerge the flat surface is directed backwards. It will be observed that the cells on the convex side are somewhat rectangular in outline and much smaller than the tall columnar cells on the flat side. In the upper part of the section where the convex part of the embryo abuts on the flat side there is an abrupt passage from the rectangular to the columnar type of

cell. This spot can be recognised in later stages, and will be denominated *x*. In the embryo under consideration a cell can be seen at this point whose nucleus exhibits dot-like aggregations of chromatin, indicative of one of the prophases of mitosis. Another case of mitosis can be seen in the middle of the convex surface. When we examine the lower border of the section at the point marked *y* in the figure where the convex surface passes into the flat surface, we find that there is a gradual transition from the rectangular to the columnar type of cell. If we now examine the nuclei we find that in all the cells they are of a uniform character so far as their staining properties are concerned. They present a vesicular appearance, the nuclear sap being crossed with cords of linin on which are very minute chromatin granules. Turning our attention now to the granules of yolk we find that these are most numerous in the large cells of the flat side and become fewer as we pass to the cells of the convex side, and that there appear in these latter cells smaller granules amongst the larger ones, but that there is no difference whatever either in size or in staining properties between the largest yolk-granules in the cells of the convex side of the blastula and those in the cells on the flat side. In this stage of development, therefore, it is impossible to say that the cells of the embryo are segregated into two definite tissues, as Lwoff and Cerfontaine have maintained, for as we have already remarked, and as a simple inspection of the figure will prove, the cells of the flat side pass by insensible gradations into those of the convex side. If with these authors we call the cells of the convex side ectoderm and those of the flat side endoderm, it is impossible, if we consider the point *y*, to say where the one ends and the other begins. At the point *x* there is an abrupt change in the size of the cells, and this is manifestly due to the beginning of a process of division by which the size of the cells is reduced. The smaller yolk-granules which appear amongst the larger ones in the cells of the convex side are due to the utilisation and fragmentation of these owing to the process of multiplication of cells which has just taken

place, and of which the mitosis shown (*k*) is evidence. A slightly later stage is shown in Pl. 1, fig. 2. In this embryo the process of invagination has commenced. At the point *x* we may recognise the same abrupt transition in the size of the cells which we remarked at the same place in the preceding stage. Here an unmistakable mitosis can be seen. The impression conveyed to the mind is that the new cells are added to the flat side by the process of cell division which has begun at *x*, and that these have exercised a lateral strain to which the flat side has yielded by bending inwards. The convex side has become more convex than in the preceding stage, and its cells more numerous. The cell-division at *x* has therefore probably added cells also to the convex side, but there is not the slightest evidence that any cell originally on the convex side has passed on to the flat side or vice versa.

In fig. 3 we have a median sagittal section of a still later stage. We again notice a mitosis at the point *x*. We obtain further evidence that the cell division which is taking place at this point does not result in a passage of cells from the convex side to the invaginated side of the embryo, because the cells adjacent to this point on the two sides are exceedingly different from one another. Those belonging to the convex side are smaller than those on the invaginated side, and have fewer yolk-granules. The new cells on the convex side are remarkable for their rounded form; cells of this shape have been noticed in the neighbourhood of the blastopore by several authors; the shape seems to be indicative of the state immediately following division when the centric force of the new nucleus is at its maximum strength (v. Théel's beautiful figures of the development of *Echinocyamus pusillus* [33]). At the point *y* the large clumsy cells are continued some little distance beyond the lip of the invagination on to the convex surface.

A still later stage is represented in fig. 4. As compared with fig. 3, we note that the invagination has become deeper, and that a decided difference in staining property between the nuclei belonging to the convex side

and those belonging to the invaginated area is now observable. The former stain more deeply, since their nuclear sap has to some extent acquired staining powers. The transition between lightly staining and more deeply staining nuclei takes place exactly at x , but at the lower lip of the blastopore the large vesicular nuclei are continued round for a short distance on to the convex side of the embryo. I regard this difference in staining power as the outward sign of the physiological differentiation of the cells into ectoderm and endoderm; it is observable all through the later stages of gastrulation and during the formation of the mesoderm. I conclude that at the point x we have a growing centre, comparable to the meristem of a root or the cambium of a tree with secondary growth, whence on the one side young ectoderm, and on the other side young endoderm cells are produced. To the appearance of this centre of growth I attribute the first impulse towards gastrulation, and the push which causes the in-bending of the lower cells should on this view be directed not towards the centre of the lower surface of the flattened blastula, but more towards its dorsal edge. As a result the invagination does not at first involve all the cells which will eventually be included within the archenteron, but the endodermal vesicular nuclei extend beyond the lower lip of the blastopore at y . A mitosis can be seen in fig. 4 in the centre of the convex surface, and also one at the apex of the invaginated area, and they serve to emphasise the fact that division of cells is not by any means confined to the growing point x . According to this view the ectodermal nuclei become differentiated from a type of nucleus which we may call endodermal, and this is in accordance with what we should expect, for the type of nucleus found in an assimilative cell must be the primitive one.

In figs. 5*a* and 5*b* we have two sections from a sagittal series through an embryo almost of the same age as, or very little older than, that represented in fig. 4. Fig. 5*a* represents a median, fig. 5*b* a more lateral section. In both figures the sharp contrast between ectodermal and endodermal

nuclei is seen, but fig. 5*a* deserves our special attention because of the small rounded cells which are seen on both sides of the point *x*. As may be clearly seen by reference to Cerfontaine's paper, it was on sections like this that that author based his conclusion that ectodermal cells were being added to the vault of the archenteron; we have already suggested that the rounded form is a passing phase following on mitosis; now our figure shows that the rounded cells which are added to the archenteric wall have vesicular nuclei, whilst those added to the ectoderm have more deeply staining nuclei; all question, therefore, of there being any "inflection" of ectoderm cells is settled in the negative. I have spoken of "the point" *x* when referring to a median sagittal section of the gastrula; the growing point is really, however, a horizontal arc, and appears in several sections on each side of the median one; fig. 5*b*, which represents a lateral section in which the cells at the dorsal lip of the blastopore are quiescent, lies four or five sections to one side of the median one. The ectoderm cells immediately beyond the growing point in fig. 5*a* are seen to be specially tall and columnar. This is the first indication of the nerve-plate (*n.p.*), and is of the greatest importance for the decision as to the orientation of the blastopore. It is also seen in the older stage represented in fig. 6 (*n.p.*); from these two figures we may conclude that the diameter of the wide-open blastopore is at right angles to the long axis of the nerve-plate, and this conclusion seems to me to settle in the negative the question of the closing of the blastopore by a concrescence proceeding from in front backwards. For such a theory, whether advanced by Hatschek, Legros, or Cerfontaine, has always assumed that the nerve-plate is formed *pari passu* as the blastopore closes, and this could only be possible if the long axis of the nerve-plate and the diameter of the blastopore coincided in direction. In fig. 6 we can see several mitoses in what even Cerfontaine would admit to be the endodermic portion of the archenteron, and, what is most interesting, one of the cells

undergoing mitosis shows the same rounded form, which, when occurring at the dorsal lip of the blastopore, Cerfontaine regarded as an infallible proof of ectodermal origin.

In figs. 7 *a* and 7 *b* (Pl. 2) a median and a lateral section of a still older embryo are represented. The nerve-plate is now unmistakably present, as the flattened dorsal surface of the embryo shows. The abrupt passage from ectodermal to endodermal nuclei at the point *x* is also evident. Now, however, we may see the extent of the histological difference between the cells forming the roof and those forming the floor of the archenteron, on which Lwoff and Cerfontaine lay so much stress, and which has also been referred to Morgan and Hazen. The yolk-granules in the cells of the roof (*ch.*) are becoming fewer, and there are mixed with the large ones smaller granules, and they do not stain quite as deeply as those in the cells forming the apex or anterior end of the archenteron. All these appearances are due to one cause; the yolk-granules are being more rapidly used up in the cells forming the roof of the archenteron than elsewhere, and this in turn can be referred to two causes: first, these cells have undergone more rapid multiplication than those forming the rest of the archenteron; and secondly, it is from these cells that the notochord will eventually be formed, and the incipient disappearance of the yolk-granules may be in part an anticipation of that vacuolisation which distinguishes the notochordal cells from those forming the rest of the archenteron. It is in any case much less observable in the more lateral section represented in fig. 7 *b*.

We have now traced the development of the gastrula step by step from the stage of the flattened blastula up to what we may term the thimble stage, in which the nerve-plate is clearly developed and about the orientation of which there can be no possible doubt, and I consider that this is the best answer to the charge of Morgan and Hazen that I confounded the two lips of the blastopore. No such close series of stages is represented in their paper. The gastrula, whilst still retaining its wide-open blastopore, has become deeper and

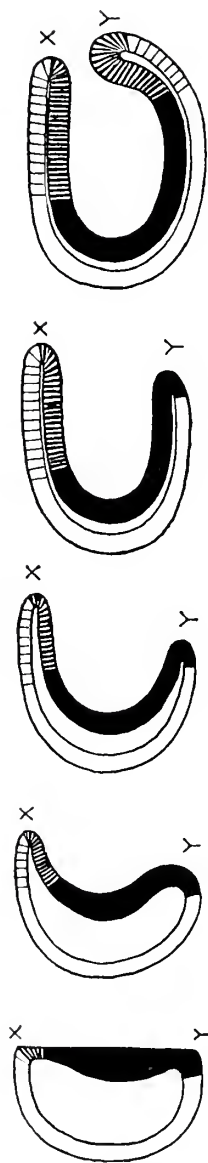
more cylindrical, due in all probability to a widely distributed increase of cells both in ectoderm and endoderm. This is the beginning of that persistent growth in length which converts the short plump embryo into the comparatively long attenuated two-day-old larva. The axis of the nerve-plate is clearly at right angles to the diameter of the blastopore, and how that can be reconciled with a theory of concrescence of the dorso-lateral lips of that aperture I leave it to advocates of that theory to settle.

The last stage in the gastrulation with which I shall deal is represented in fig. 8. Here we see the process of closing, or more strictly speaking of narrowing the blastopore. A centre of growth and multiplication of cells has now appeared in the ventral lip of the blastopore, and in consequence this lip has grown upwards and protrudes as a rounded mass blocking the cavity of the blastopore. We are forcibly reminded of the yolk-plug in Amphibian embryos; and at the same time we note that all the cells with vesicular nuclei are being brought within the confines of the archenteron. It was the discovery of this new centre of growth that led Cerfontaine to imagine that in the later stages of gastrulation ectodermal cells were being inflected at the ventral lip of the blastopore; it will be seen also that Klaatsch was perfectly justified in supposing that cells passed in at this lip, only the cells that passed in are endoderm, not ectoderm.

The remnant of the blastopore is eventually found as a small pore at the posterior end of the nerve-plate, in which position it becomes covered in by the meeting above it of the ectodermal folds which arise at the sides of this plate.

Figs. 9 and 10 represent sections from series cut through nearly complete gastrulae in a horizontal direction, in order that some account may be taken of the condition of the "lateral" lips of the blastopore. Fig. 9 is a section from the middle of the series cutting the blastopore near the mid-horizontal line, whilst fig. 10 is a section which cuts the blastopore near the dorsal lip. In both we see the sharp

TEXT-FIG. 1.



1.

The gastrulation of *Amphioxus*. The darkest tint represents the original endoderm; the close-barred dusky shade the new endoderm added by the growing points. White is original ectoderm, loose-barred new ectoderm.

contrast between the ectodermal and endodermal nuclei at the lips of the blastopore, but in the more ventral section the endodermal nuclei protrude beyond the lip somewhat, whereas in the more dorsal they are pushed inwards by the overgrowth of ectoderm. From these figures we may conclude that the point *y* like the point *x* is really an arc of considerable lateral extent—in fact, of much greater extent than *x*. We should probably be justified in dividing the whole circumference of the blastopore into a dorsal and a ventral arc; by cell division in the former gastrulation is initiated, by cell division in the latter the blastopore is closed. Text-fig. 1 represents approximately my view of the steps by which gastrulation proceeds in *Amphioxus*. It is impossible, of course, to define the limits of the new endoderm and of the original endoderm which formed the flat surface of the hemispherical blastula, because, as we have seen, mitoses take place in the original endoderm and thus it increases in extent. The text-fig. gives the most probable view of their relative extent. On account of this cell-division in the original endoderm its cells become reduced in size, and at the conclusion of gastrulation the archenteron is lined by a layer of cells which are all of approximately equal size.

THE FORMATION OF THE NOTOCHORD AND OF THE MESODERM.

We have seen that the blastopore becomes covered in by the meeting above it of two folds of ectoderm, which arise at the sides of the nerve-plate. These folds first arise in the hinder part of the embryo and first meet there, and they then grow gradually forwards, but for some time the anterior part of the nerve-plate is uncovered. During this time the embryo is growing in length and *pari passu* diminishing in diameter. The only possible explanation of this phenomenon which I can make to myself is that the yolk-granules are being used up, and that coincidently the cells are multiplying all over the animal, but especially towards the hinder end. If we

now examine a series of transverse sections through an embryo in which the diminution in diameter is only slight, and in which a good part of the nerve-plate is still uncovered, we shall see the first stages in the formation of the mesoderm and the notochord. As my contention that in the formation of its mesoderm *Amphioxus* resembles *Balanoglossus* is based on an examination of this stage, and as Cerfontaine appears to have completely missed it, I have thought it wise to figure in detail five sections from such a series. Figs. 11 *a, b, c, d,* and *e* (Pl. 2) represent these sections. In fig. 11 *a*, the most anterior, we see the nerve-plate consisting as yet of a quite horizontal platform of columnar ectoderm cells. In the centre are two clear cells (*oc*), which I suspect are part of the rudiment of the eye-spot which is found in this position in later larvæ. The archenteron shows at its dorso-lateral angles two pouch-like outgrowths (*coll.*). The form of these pouches and the sharp angle at which their cavities unite with the cavity of the archenteron negatives any idea that they could be due to a simple process of folding of the archenteric wall; they are clearly due to an active process of proliferation which takes place at this position in the endoderm. In fig. 11 *b*, which is only two sections further back, we see that the nerve-plate is no longer flat but gently arched inwards, and the beginnings of the two ectodermic folds (*f*), which are later to meet above it, are seen at its sides. In the archenteric wall we observe the same pouches that we remarked in the more anterior section, but above them and situated nearer the mid-dorsal line we find another pair of similar pouches (*tr.*). In fig. 11 *c*, which is only two sections further back still, the ectodermic folds have almost, but not quite, met above the nerve-plate, and the first and more ventral pair of pouches of the archenteric wall have disappeared, but the second or more dorsal pair persist and continue through half a dozen sections more, always open to the gut, though this opening is narrower in one place than it is in front or behind. Now the first or more ventral pair of pouches give rise to the first pair of somites, whilst the

more posterior and dorsal pouches give rise to all the other somites. The first pair of somites therefore stand in a different category to all the rest. This is true not only of their origin as independent evaginations of the archenteric wall, but as we shall presently see of all their subsequent history. I compared these somites in 1898 to the collar cavities of *Balanoglossus*, and all my subsequent research has confirmed me in this view. The more posterior pair of pouches will then be equivalent to the trunk cavities of *Balanoglossus*, and the varying diameter which these show as we follow them backwards through this series is the first indication of their approaching division into the posterior somites, which is the great point in which *Amphioxus* exhibits an advance on the condition represented by *Balanoglossus*. In the stage under consideration there seems to be a constriction of the trunk cavity in one place, but throughout its whole extent it is freely open to the gut. This constriction is an indication of the constriction of the first of the posterior somites from the front end of the trunk cavity. In fig. 11 *c* we observe in the mid-dorsal line of the archenteron the first indication of the formation of the notochord (*ch.*). The archenteric wall is here arched slightly upwards, and the yolk-granules are being rapidly reduced in number so that the cells have become relatively clear. Here we have the explanation of that difference between the roof and the floor of the archenteron as seen in median sagittal sections, on which Lwoff and Cerfontaine founded such top-heavy conclusions. Fig. 11 *d* is taken from near the hind end of the embryo. The nerve-plate is completely covered in by the union of the ectodermic folds above it, so that we now have a neural canal (*n.c.*). The archenteron shows a division into two storeys, exactly as Legros has described for his abnormal embryos, but which is here described for the first time in a normal embryo at least of this age. The lower division (*a*) is the anal diverticulum, which at a much later stage will open to the exterior. The upper division (*a'*) leads to the still open

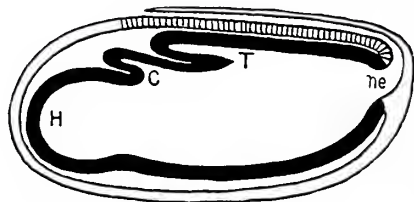
blastopore, which lies beneath the cover of the ectodermic folds and forms a space at the hinder end of the animal into which the neural canal also opens, viz. the neurenteric canal. This is shown in fig. 11 *e* (*ne.*) two sections further back in the series. In fig. 11 *d* we see on the mid-dorsal wall of the archenteron a band of cells undergoing mitosis. According to Legros and Cerfontaine the notochord, and the nerve-plate too for that matter, are formed by the union in the middle-line of two moieties. There is certainly no trace of such a process here for the band of dividing cells is continuous across the middle-line. Of course in every structure there is an imaginary middle-line, and if anyone chooses to say that this band of dividing cells consists of right and left halves which unite together as quickly as they grow, I shall not waste time in arguing against such a metaphysical conception, which is capable neither of proof nor disproof. Legros further asserts that the horizontal ridges which he finds in the sides of the archenteron in his abnormal embryo mark the limits of the ectodermic and endodermic portions of this structure. A glance at fig. 11 *e* will show the futility of such a conception; the histological character of the cells forming the upper and the lower portions of the archenteron is identical, whereas the place where the ectoderm abuts on the tissue of the archenteric wall is clearly seen. But, indeed, Legros's own figures, schematic as they are, refute this contention, for they show the genuine ectoderm to be widely different from the so-called ectoderm forming the roof of the archenteron. About this stage or a little later the embryo escapes from the egg-membrane and becomes a free-swimming larva, propelling itself by its ciliated ectoderm. In figs. 12 *a*, *b*, *c*, and *d* (Pl. 3) four sections through a somewhat older embryo are represented, and its smaller diameter, when compared with the embryo which we have just considered, should be noted (*vide* the magnifications given in the description of the plates). The union of the ectodermic folds above the nerve-plate has proceeded forward, and is seen to be complete in the most anterior section. In this section we see the two

collar-cavities still in open communication with the gut, and the beginning of the notochord is indicated between them, showing that the formation of this structure has also proceeded in an anterior direction. In fig. 12 *b* on the left side the collar-cavity is seen to be closed off from the cavity of the archenteron, and lying beside it nearer the middle line is the trunk-cavity equally shut off from the archenteron. In fig. 12 *c* the dying out of the collar-cavity can be seen on the left side, whilst on the right side the collar-cavity can be seen to be closed off from the archenteron. Finally in fig. 12 *d*, which is taken from considerably further back in the series, the hinder portions of the trunk-cavities or trunk-folds can be seen opening freely into the archenteron. It follows that the anterior portions of the collar-cavities and the posterior portions of the trunk-cavities retain their openings into the archenteron. The trunk-cavity has already had two somites cut off from it. It will be noticed that the formation of the notochord is most advanced in those sections in which the collar-cavities are visible, recalling the conditions found in *Balanoglossus*.

The first indication of the head-cavities is seen when five somites have been cut off from the anterior part of the trunk-cavity. A section through the front part of such a larva is shown in fig. 13. The notochord (*ch.*), which by this time is completely detached from the archenteric wall, has grown forward towards the extreme front end of the embryo. Above it we see the nerve-plate grooved and partly covered by an ectodermic fold, leaving, however, a pore—the neuropore—which persists for a long time in the free-swimming larva. The archenteron may be seen to be produced above into a thin bi-lobed vesicle. This is the rudiment of the two head-cavities, and may be compared to the anterior outgrowth which gives rise to the proboscis cavity in *Balanoglossus*. Already the left lobe may be seen to have a thicker wall than that on the right side. In fig. 14 a section through an older larva in the same region is shown. In this embryo six somites have been cut off from the front end of the trunk

cavity. By the forward growth of the nerve-plate the neuropore region has been carried forwards, so that in the section figured it is no longer seen, and not only have the ectodermic folds met above the nerve-plate, but the nerve-plate itself has been bent into a nerve-tube. The notochord and the anterior portions of the collar-cavities have shared in this forward growth, and these last are seen above the archenteron at the sides of the notochord. Turning our attention now to the archenteron we observe that a right thin-walled vesicle and a left thick-walled one are in process of being shut off from a central thick-walled gut, but that there is still a narrow seam

TEXT-FIG. 2.



2.

Illustrating the formation of the mesoderm in *Amphioxus*. H. Head-cavity. C. Collar-cavity. T. Trunk-cavity. ne. Neurenteric canal.

of communication between right and left moieties. A little later these are completely separated from the definitive gut and from each other. The relationship to one another of head, collar and trunk-cavities is schematically represented in text-fig. 2, in which an *Amphioxus* embryo is supposed to be seen from the side. The embryo is supposed to be transparent, and the formation of the head-cavities is antedated so as to make it contemporaneous with that of the collar and trunk-cavities. The portion of the gut, however, from which the head-cavities are eventually formed can be clearly seen in even young embryos, such as that figured in figs. 11 *a-d*. It projects in front of the region from which the collar-cavities are eventually formed.

The later history of these cœlomic vesicles and of the rest of the archenteron which constitutes the definitive gut may be followed in larvæ, in which the mouth is about to be formed and the first gill-slit about to break through. This stage is almost the latest to which it is possible to rear the eggs artificially; for older larvæ one has to depend on the tow-net. A series of seven sections through such an embryo is represented in figs. 16 *a-g*, fig. 16 *a* being, of course, the most anterior section. In this section the neuropore is seen and the notochord lying beneath the nerve-cord. At the sides of the notochord the slit-like anterior ends of the collar-cavities can be seen, whilst beneath the notochord is seen the thin-walled right head-cavity which has become shifted anteriorly to its left fellow. This is one of the first signs of that curious asymmetry which is one of the peculiarities of *Amphioxus* larvæ, and which has so puzzled all investigators. A satisfactory, or at any rate, a plausible explanation of it has been suggested to me and will be given later on. In the more posterior section fig. 16 *b*, the left head-cavity is seen lying above the right one as a completely closed thick-walled vesicle. A vacuolated ectodermal cell (*v.*) marks the spot where this vesicle will later acquire an opening to the exterior. This section therefore completely refutes the view of Legros (23) that this vesicle is an ectodermal invagination. Van Wijhe (35) had already decided in my favour after examining preparations sent him by both Legros and myself. The nerve-tube is closed and at the sides of the notochord the collar-cavities are seen as spacious cavities, the inner walls of which are, however, becoming thickened. In fig. 16 *c* the anterior blind end of the gut is grazed, and in the thickened inner walls of the collar-cavities adjacent to the notochord numerous muscular fibrils (*musc.*) are seen—these being the first traces of the formation of the first myotome. It is of particular importance to note that the lower parts of the collar-cavities which are wedged in between the ectoderm and the gut are in open communication with the upper myotomic portions. The cavity on the right side extends

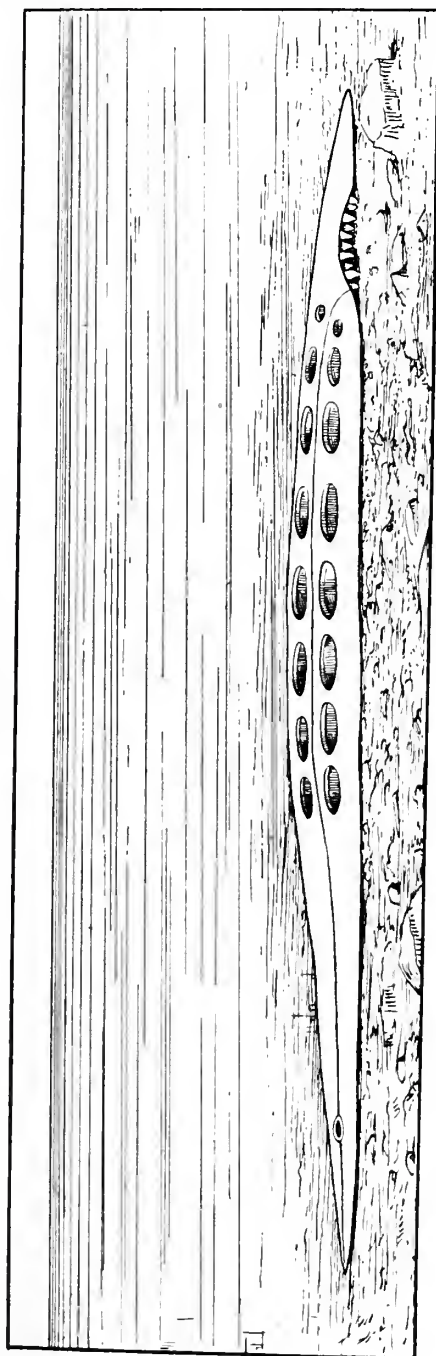
nearer the mid-ventral line than the cavity on the left side. Below the most ventral point of the latter the gut is pressed against the ectoderm, which here exhibits a plate of large cells; this plate marks the place where the perforation to form the mouth will take place. In the next section, shown in fig. 16 *d*, the enlargement of the anterior end of the gut to form the pharynx is clearly seen, and on the right side of this there is a pouch-like outgrowth, the rudiment of the club-shaped gland. This structure has been interpreted by Willey (36) as the fellow of the first gill-slit, and in this opinion I am prepared to concur. The posterior somites which have resulted from the segmentation of the collar-cavity are seen to be completely divided into myotome (*my.*) above and splanchnoceles (*spl.*) below, but beneath and external to the right and left rudiments of the splanchnoceles are to be seen the posterior prolongations of the right and left collar-cavities (*r. coll., l. coll.*). These extend just as far back and no further than the enlargement of the gut, which is the rudiment of the pharynx. In the next section, fig. 16 *e*, the same features are observable, but here we find the first gill-slit about to open, and we see that as in all Vertebrata it is produced by the meeting of an endodermal outgrowth (*br. end.*) and an ectodermal ingrowth (*br. ect.*). Figs. 16 *f* and *g* are two sections from the most posterior part of the series. In fig. 16 *f* we see the anal diverticulum (*a.*) which we had already noted in a much younger embryo, but the anus is not as yet open. In fig. 16 *g*, two sections further back, we see the solid cord of cells uniting gut and nerve-cord (*nc.*), which is the representative of the neurenteric canal of earlier forms, and this acts as a "meristem" for the tail, which in *Amphioxus* is of relatively small dimensions. Since the division of the gut into two storeys is still near the hind end of the animal, in spite of the great growth in length which has taken place, we draw the conclusion that this growth must take place mainly in front of this region, and that the band of actively growing

cells is therefore situated just about where it is in the embryo represented in fig. 11. Throughout the sections of this series the modifications which the notochordal cells undergo may be clearly seen; we can perceive that the greater part of each cell becomes converted into a vacuole, but that the nucleus and a piece of the protoplasm immediately surrounding it persists. In the old free-swimming larvæ a peculiar tube, termed Hatschek's nephridium, can be seen lying above the front end of the pharynx and opening into it posteriorly. In 1898 I asserted that this tube was the persistent connection between the left collar-cavity and the pharynx. This connection lasts certainly for a long time, and much longer on the left side than on the right, and it is found in the same place as Hatschek's nephridium. It is shown in an embryo of the same age as the one we have been considering in fig. 15 as a cord of cells with virtual cavity connecting pharynx and left collar-cavity (*neph.*). Owing, however, to the gap which in my material intervenes between larvæ of this age and those in which an undoubted Hatschek's nephridium is present, I am not prepared to dogmatically maintain my assertion of 1898. The oldest stage to which I shall refer in this paper is represented by fig. 17 *a-h* (Pl. 5) inclusive, which represents eight sections taken from the same transverse series. The specimen which was sectioned was a larva with the mouth and first gill-slit open and the club-shaped gland fully formed. In the most anterior section (fig. 17 *a*) the left head-cavity (*lh.*) is shown to have developed an opening to the exterior. In fig. 17 *b* the external opening of the club-shaped gland is shown lying just ventral to the thickened plate of cells which forms the anterior border of the mouth. In fig. 17 *c* the mouth is seen opening on the left side of the larva and the cavity of the club-shaped gland can be seen on the right side of the pharynx. Beneath the right rudiment of the splanchnoceles the right collar-cavity can be seen, and the ectoderm surrounding it is thickened—this is the first visible rudiment of the atrial ridge (*at.*), which eventually meets its fellow so as to enclose the atrial cavity. Fig. 17 *d-h*

represents a series of five consecutive sections through the hinder end of the pharynx to show how the collar-cavities and the pharynx cease simultaneously, and that at the same time the right and left rudiments of the splanchnoceles meet in the mid-ventral line. If we examine the hinder end of the pharynx in a much older larva a similar set of appearances can be made out. Hence we conclude that as new gill-slits are added in the extraordinary manner described by Willey (37) the pharynx grows in length, and *pari passu* the collar-cavities and their enclosing atrial ridges which flank it. Van Wijhe (34) has described in the adult animals cavities in the edges of the oral hood and in the sides of the atrial cavity, which he identifies with these inferior portions of the collar-cavities. He believes, however, that in the adult the upper portions of the collar-cavities become divided into several myotomes. His conclusions are very interesting, but owing to the lack of material of older stages on which I can depend I cannot either confirm or combat his assertions. I think, however, that my explanation of the left-sided mouth is simpler than his.

I said above that a plausible explanation of the asymmetry of the *Amphioxus* larvæ, which has proved a puzzle to most investigators, had been suggested to me. The credit of this is due to my assistant, J. Stafford, Esq., Ph.D., but as he is unwilling to publish I give it here and take full responsibility for it. It is as follows: The pelagic larva of *Amphioxus* represents a pelagic ancestral condition of the race. Whilst in this condition the ancestors of *Amphioxus* were fully bilaterally symmetrical, and from this stock the rest of Vertebrata are descended. The adult *Amphioxus* leads a burrowing life in sand, a degenerate condition of affairs compared with its former free-living condition. Nevertheless, in both conditions of life the food and the mode of obtaining it are the same. By ciliary action small free-swimming organisms are whisked into the mouth, and the surplus water escapes by the gill-slits. Now *Amphioxus*, like the majority of fish, is a laterally compressed animal, taller than it is

TEXT-FIG. 3.



3.

The "Pleuronectid" ancestor of *Amphioxus* lying on its left side. The line running along the belly of the animal is the morphological mid-ventral line.

broad, and can only maintain its equilibrium so long as it moves. When it ceases moving it falls on one side, and this is true of the larvæ of *Amphioxus*, as Willey has pointed out (37). Between the condition of life when the ancestors of *Amphioxus* were entirely pelagic and the condition in which they burrowed, there intervened in all probability a transitional condition when they lay on their left side on the bottom, making only occasional excursions through the water. In this "Pleuronectid stage," as we may call it, which in all probability represents the mode of life of the older *Amphioxus* larvæ, it would be advantageous to twist the originally mid-ventral mouth on to the left side so as to improve its opportunities of feeding, and at the same time to twist the left gill-slits on to the upper right side so as to remove them from the substratum and place them in a favourable position for evacuating water.

The twist of the mouth seems to have been effected by a greater growth of the right side of the animal, and hence it comes about that the endostyle is at first anterior to the mouth, and so is also the opening of the club-shaped gland (fig. 17 *b*). But in the region of the gill-slits the opposite kind of an inequality of growth must have subsisted, for by greater growth of the left side the left gill-slits are pushed on to the right side, and the mid-ventral line is displaced high up on the right side. Very similar conditions prevail in the head of a developing sole, for in this animal the mouth is twisted downwards and the left eye upwards. In the ontogeny of the individual, however, these inequalities in growth have been hurried on before their time, and the reason why the left gill-slits only (Willey's primary gill-slits) are found in young larvæ is that in larvæ of small size, when there ought to be a number of similar structures performing the same office, Nature reduces the number owing to considerations of want of space (cf. the Nauplius larva of Crustacea with only three pairs of legs, and the single lateral cerebral eye in the Ascidian tadpole).

Van Wijhe (35) has given a somewhat similar explanation.

He supposes that in one stage of its history *Amphioxus* swam in spirals, turning ever from right to left. Under these circumstances he supposes that the first gill-slit of the left side, corresponding to the left spiracle of *Elasmobranchii*, became enlarged so as to function as a new mouth. He identifies the present mouth with the spiracle, because he supposes that the collar-cavity passes down under it and not over it, and he quite justly identifies the collar-cavity with the mandibular cavity of *Elasmobranch* embryos. In the supposition that the mouth of *Amphioxus* lies behind the lower extension of the collar-cavity he is mistaken, as I hope the present paper will convince him. I am sorry to differ from Van Wijhe, for whose generous recognition of my results I am grateful, but I think that my theory of the twist of a pre-existing mouth is preferable to any theory which postulates the formation of a new one, and more in line with what we know to have occurred in other divisions of the animal kingdom.

We may sum up our general survey of the formation of mesoderm and notochord in *Amphioxus* thus: Both mesoderm and notochord in *Amphioxus* are of ENDODERMAL origin, and the mesoderm origiuates in a manner closely recalling that which Bateson described for *Balanoglossus*; the differences being that the proboscis cavity divides into two completely separate head-cavities, that the collar-cavities have long antero-dorsal and postero-ventral extensions, are in a word obliquely orientated, and that the trunk-cavities become broken up into a series of somites, the dorsal portions of which form muscular masses, whilst the more ventral portions form an undivided splanchnoceles. The notochord, as in *Balanoglossus*, becomes marked first in the collar region (see fig. 12), and grows forward into the head region subsequently. The hindermost part of the gut divides into neurenteric canal and anal diverticulum.

PART II.

COMPARISON OF THE PROCESS OF THE FORMATION OF THE
LAYERS IN AMPHIOXUS WITH THE SAME PROCESS IN THE
HIGHER VERTEBRATA.

Before entering on a comparison of the formation of the layers in *Amphioxus* with the same process amongst the higher Vertebrata, it is necessary to discuss the position of *Amphioxus* amongst the Vertebrata. Hubrecht, and some others with him, have argued that *Amphioxus* is not a primitive but a degenerate form, and hence that its development must be disregarded, for this would prove exceedingly inconvenient for their theories. Now it must be admitted that in some points *Amphioxus* is degenerate, and, as we have seen, its degeneration may be brought into connection with its burrowing habits in which it has diverged from the life of its class. But the same is true of all the primitive forms which Nature has kindly preserved for our inspection, except those few, like the *Sphenodon* of New Zealand, which are confined to very restricted and sheltered areas. All widespread primitive forms must be degenerate, for how could a primitive animal, whilst retaining its ancestral habits, maintain itself in competition with the improved races which have sprung from its stock? The thing is absurd; such an animal can only maintain itself by getting out of the way, and taking to a secluded mode of life. Moreover, if *Amphioxus* is objected to on this ground, an equal objection must be sustained against *Peripatus*, against *Chiton* and the *Amphineura* amongst Mollusca, against *Limulus* amongst Arachnida, against, in fact, every animal that has thrown any light on the primitive structure of the class to which it belongs. All workers in vertebrate morphology, when they have to deal with the evolution of particular organs, descend to *Amphioxus*; and why should we not have recourse to this form when we seek to give an account of the formation

of the layers, seeing that it is precisely in respect to this process that the modifications induced by the adaptation of the stock to a burrowing life do not make themselves felt. The egg of *Amphioxus* is relatively free from the factor that produces the greatest amount of disturbance in the development of the eggs of the higher Vertebrata, viz. food-yolk. Moreover, it has an extremely short embryonic life within the shelter of the egg-shell and a long larval existence; and a long larval existence is everywhere a primitive condition of affairs. As Korschelt and Heider in their 'Text-book of Embryology' judicially remark, reproduction by ciliated larvæ cannot be interpreted otherwise than as a primitive feature. But the best proof of all, that the development of *Amphioxus* is really primitive, is to be found in the way it lends itself to the harmonious explanation of the development of the higher Vertebrata, assuming as a modifying factor only the disturbing influence of food-yolk, as I shall now endeavour to set forth.

In our search for other types of development amongst Vertebrata which may most readily be compared with that of *Amphioxus*, there is one most important consideration to be borne in mind, viz. that a very large amount of material of the earliest stages of development is needed for a successful analysis of the process of formation of the layers. The closer to one another are the successive stages available to the investigator, the less likelihood of error will there be in his results. Such a condition of affairs is possible in the case of very few types of Vertebrata. In reviewing the list of Vertebrata which have been studied, we see that this condition is fulfilled only in the case of Ascidiæ, of Urodela and Anura amongst Amphibia, and to a lesser extent of Cyclostomata, Dipnoi, Ganoidei and Teleostei amongst fishes, but these piscine groups have not been studied with anything like the care that has been lavished on Ascidiæ and Amphibia.

In these two last-named groups thousands of eggs of every assignable stage can be easily obtained and the processes

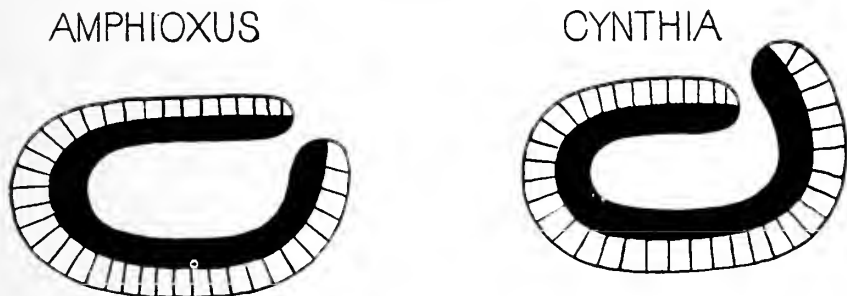
thus followed step by step. But contrast with this the conditions offered by the development of Elasmobranchs, Reptiles and Mammals! Here an adult animal has to be sacrificed to gain a very small number of eggs; and a long series of years is requisite before anything like a continuous series of stages can be obtained. A great many of the difficulties and disputes which have arisen in connection with the interpretation of the early developmental stages of the higher Vertebrata are due to this cause alone. Again, if the theory of evolution be true at all, the developmental stages of the lower Vertebrata should be less modified than those of the higher, and should contain the key to them. To quote the 'Text-book of Embryology' of the late Professor F. M. Balfour on the subject of the development of the fowl, "the subject itself is by no means commensurate with the attention it has received. The characters which belong to the formation of the layers in Sauropsida are secondarily derived from those in Ichthyopsida, and are of but little importance for the general questions which concern the nature and origin of the germinal layers." It is therefore amazing to find Hübner advising students of embryology to tackle Amphibia rather than Amphioxus, and mammals rather than cartilaginous fishes. The embryology of the higher forms may show how the process of the formation of germinal layers may be modified, but not how it originated.

Under these circumstances we may first turn to the development of the simple Ascidians. The figures given by van Beneden and Julin for *Clavellina* and by Kowalevsky for *Ascidia*, as reproduced by Korschelt and Heider, are strikingly like the figures I have given above for the gastrulation of *Amphioxus*. We see the advance of the dorsal anterior lip of the blastopore, and the coincident formation of the neural plate above it, and then in the second stage the up-growth of the lateral and ventral lips and consequent narrowing of the blastopore. The main differences are: (1) the contrast between endoderm and ectoderm cells is greater than in *Amphioxus*, and the whole number of cells in both layers is smaller and the

individual cells are larger; (2) the up-growth of the ventral lip is more marked and takes place at an earlier stage.

The whole subject has been fully dealt with by that able student of cell lineage, Conklin, in his exhaustive description of the development of *Cynthia* (19), where he has accounted for every individual cell and shown its lineage. Conklin emphatically states that the closing of the blastopore includes two stages, in the first of which the dorsal lip grows backwards, and in the second and subsequent stage the ventral lip grows upwards and the lateral lips at the same time coalesce. Conklin admits that mesoblast and notochord are

TEXT-FIG. 4.



4.

Diagrams comparing an advanced gastrula of *Amphioxus* and of *Cynthia*. (According to Conklin.)

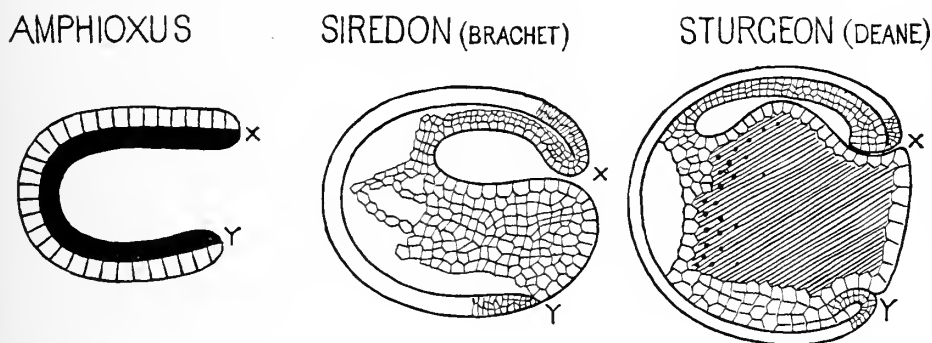
differentiated and their component cells recognisable before they are invaginated, but he utterly declines to call them ectoderm, "because they are large and full of yolk, and resemble endoderm."

When we leave the development of the Ascidian larva, which in its sense-vesicle and well-developed tail stands higher in the vertebrate scale than *Amphioxus*, the next highest stage is represented by the Cyclostome fishes, the development of which has been studied by many observers. Unfortunately none of these have been specially interested in the minute details of gastrulation, but the figures they give of both segmentation and gastrulation of the egg are extra-

ordinarily like those of Urodele and Anuran Amphibia. Since a very similar method of development is found also in such old-fashioned types of fish as the Dipnoi, Polypterus, and the sturgeon, we may with some probability conclude that this method was once universal amongst Vertebrates, and persisted till the first invasion of the land had been accomplished. A multitude of papers has appeared on the development of the Amphibia, but the careful and exhaustive work of Brachet¹ (6) on the comparison of the development of Siredon and of the frog will, I think, for some time represent the last word on the subject. Brachet finds that at the completion of segmentation endoderm and ectoderm are not delineated, that although the lower pole of the egg is occupied by large cells with large yolk-granules and devoid of pigment, whilst the upper pole is the seat of small cells with small yolk-granules and pigment, yet there is a gradual passage from the one type of cell to the other. As in *Amphioxus* the differentiation of ectoderm and endoderm accompanies a process of growth and multiplication of cells, some of the daughter-cells which result therefrom being endoderm with large yolk-granules, and others ectoderm with small yolk-granules. In this way a skin of small cells is, as it were, cut out from the surface of the large cells for a considerable distance below the equator of the egg. The edge of this advancing skin represents the point x in *Amphioxus*. The cavity of the archenteron appears first as a split within the region of undoubted endoderm cells. (This is beautifully and clearly shown in the figures which Bles gives of the development of *Xenopus* [5].) Then later the endoderm cells wander inwards in virtue of an altered cyto-taxis, the archenteric cavity simultaneously enlarges, and the segmentation cavity is obliterated. All question therefore of the roof of the archenteron being in-turned ectoderm is disposed of. The ventral lip of the blastopore makes its appearance later than the dorsal lip by a process of multiplication of cells, accompanied by differentiation of the daughters into ectoderm and endoderm, and it

slowly advances to meet the dorsal lip. This latter, after the invagination has taken place, has grown somewhat in length, and therefore in its edge as in the dorsal lip of the blastopore in *Amphioxus* is situated a growing point by which above new ectoderm and below new endoderm are produced. This has been proved up to the hilt by Assheton in his most interesting experimental studies (2). In *Siredon* the ventral part of the blastopore remains open as the anus, whilst the dorsal part is included within the medullary groove as the neurenteric canal. The

TEXT-FIG. 5.



5.

Sagittal sections through the half completed gastrulae of *Amphioxus*, *Siredon*, and *Sturgeon*.

tail forms as a new growth only after gastrulation is complete. It will be seen that, making allowance for the difference in the amount of yolk there is an extraordinary correspondence between the development of *Amphioxus*, as described in this paper, and that of *Siredon*. The early formation of the dorsal lip and the later up-growth of the ventral lip show the same sequence. The difference between the two embryos lies in the accumulation of yolk in the ventral wall of the archenteron of *Siredon*, so that the cells here are not only more numerous but more massive than those forming the dorsal wall, and therefore the difference between roof and floor of the archenteron, which is barely indicated in

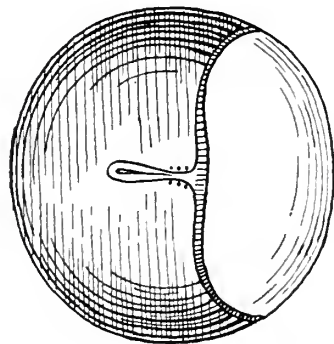
Amphioxus, is here strongly marked. This difference goes on accentuating itself as we ascend in the Vertebrate scale, and it is, of course, this difference which gave rise to the idea that notochord and mesoderm were ectodermic invaginations. The reason of this difference is not far to seek. From the dorsal wall of the archenteron notochord and mesoderm are formed by processes of folding or by proliferation. Now, for either of these two processes yolky cells are pre-eminently unsuited, and hence there is an advantage in storing the yolk necessary for development in a portion of the archenteric wall where formative processes do not occur until very late.

The work of Kerr (19) on the early stages in the development of *Lepidosiren* is based on a great quantity of material, and his figures and the general trend of his conclusions support those of Brachet. I call attention in particular to the emphasis he lays on the fact that the archenteric cavity first appears within the region occupied by the endoderm cells, and on the fact that the ventral lip of the ectoderm is "cut out" from the surface of the large cells of the ventral half of the egg—that here, as in the dorsal lip, growth with differentiation goes on.

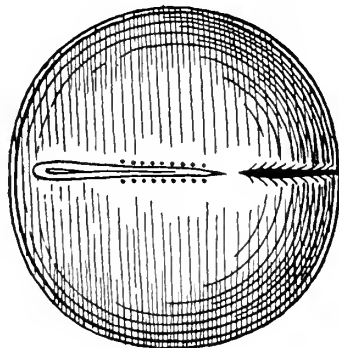
The work of Bashford Dean on the embryology of the three Ganoid fish, *Acipenser* (11), *Lepidosteus* (11), and *Amia* (12), enables us to pass gradually from a development like that of the frog to that of a meroblastic egg. In *Acipenser* (vide text-fig. 5) the whole egg is segmented superficially. Above the whole mass is divided into cells, and there is a spacious segmentation cavity below; the segmentation is superficial, and there is an interior mass of unsegmented yolk. In *Lepidosteus* there are but a few superficial furrows at the lower pole which subsequently completely disappear, whilst in *Amia* no furrows are formed at the lower pole at all. In all three cases at a certain point, which afterwards forms the dorsal lip of the blastopore, growth and differentiation of cells occur accompanied by invagination of some of the newly-formed cells. The ventral lip is formed in the same way, and grows

TEXT-FIG. 6.

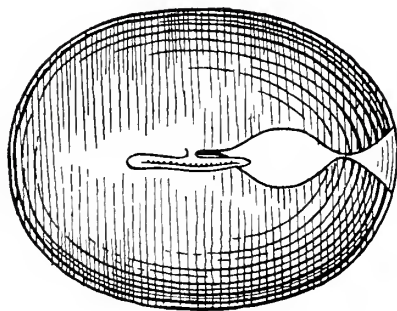
MORMYRUS



MORMYRUS



SHARK



6.

Surface views of Mormyrus after Assheton and of an Elasmobranch after Balfour.

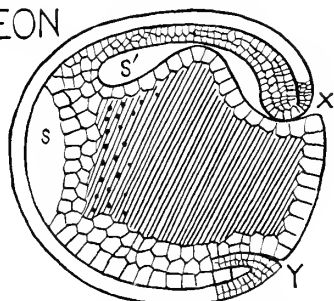
over the lower pole and eventually meets the dorsal lip and so the blastopore is closed. In none of the cases discussed can there be a question of concrescence of the lateral lips of the blastopore extending along the neural plate, because unless such concrescence is to be merely a metaphysical figment, it must mean that the dorsal lip advances by a pair of growing points situated to the right and left of the median line, whilst in the mid-dorsal line itself there is a cessation of growth. Now no such cessation is observable. A groove found in the floor of the neural canal in *Amphibia* has been interpreted as a remnant of this concrescence, but Brachet has pointed out that this groove is a late secondary phenomenon that does not make its appearance till the blastopore is closed.¹ From *Amia* we pass naturally to the development of a Teleost such as *Mormyrus*, so well described by Assheton (3). Here there is, as in *Amia* (and, of course, in all Teleostei), a well-marked germinal disc. On the surface of this the neural plate of the embryo becomes marked out by its greater thickness. But this neural plate, instead of extending as in the cases we have just discussed over 180° or more of the circumference of the egg, extends over quite a small arc, so that the embryo is really differentiated from but a small part of the egg. At the margin of the germ-disc as before we find the dorsal lip of the blastopore, but the ventral lip sweeps completely round the egg and its two sides close by lateral concrescence on the hinder aspect of what afterwards becomes the yolk-sac. After this closure has taken place, and not till then, the growth in length of the hinder part of the embryo begins. The alimentary canal becomes grooved off from the surface of the yolk-sac as a rod of cells, and the yolk-sac is slowly absorbed.

In the development of Elasmobranchs a very small germ-disc is formed on the surface of a large yolk-y egg. Inside

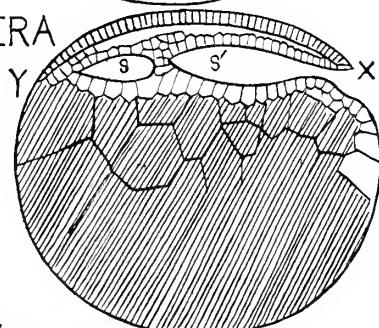
¹ It is true that Brachet in a later paper (7) admits that he is an adherent of the concrescence theory, but solely on the ground of the appearances presented by pathological embryos, which is, I think, a most illogical position.

TEXT-FIG. 7.

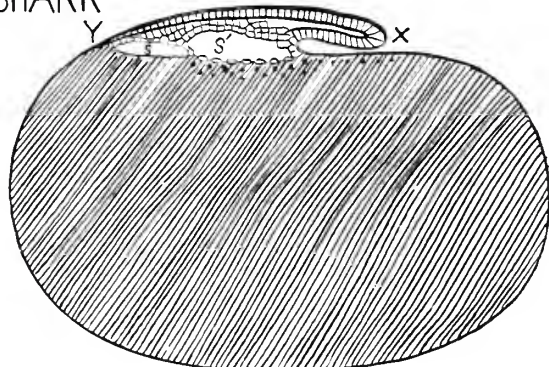
STURGEON



CHIMAERA



SHARK



7.

Median sagittal sections of the gastrulae of the Sturgeon, Chimæra and the Shark. *s*. True segmentation cavity. *s'*. Inner part of archenteric cavity sometimes formed by cleavage of the endoderm. (After Bashford Deane.)

this disc is a segmentation cavity, the floor of which at first contains some cells, but soon becomes unsegmented yolk. Growth and differentiation of cells leading to the formation of a dorsal lip of the blastopore occur as usual at the edge of the germinal disc, and at this point there takes place an invagination of the lower cells giving rise to an archenteron, the floor of which is unsegmented yolk containing some free nuclei. In *Chimæra*, as Bashford Dean has shown (13), the invagination takes place, and the dorsal lip is differentiated not at the edge of the germ-disc, but a little way inside the edge, and the yolky floor of the archenteron contains traces of cell division (see text-fig. 7). These facts point conclusively, as Dean says, to the fact that the unsegmented yolky portion of the egg corresponds, not to the whole of the large cells of the vegetable half of the frog's egg, but merely to the lower portion of these.

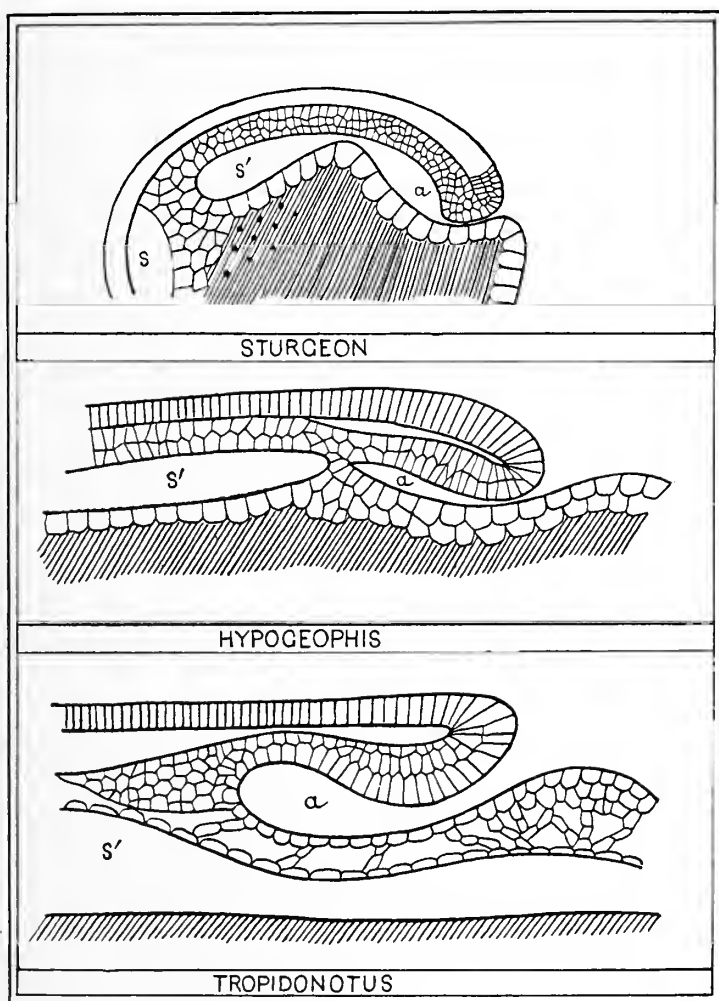
The lower layer cells of the germ-disc of an Elasmobranch correspond to the upper half of the yolky cells in the frog's egg. The ventral lip of the blastopore, however, is not, as Dean assumes, situated beneath the open blastopore. It is the free edge of the sheet of ectoderm which extends from the opposite anterior side of the germ-disc round the egg, and which eventually encloses the yolk. The Elasmobranch peculiarities are two. First, this ventral lip, instead of extending round the sphere in a great circle, extends laterally round it in a small circle, and its two sides meet behind the embryo in a linear streak before the yolk is covered (see text-fig. 6). Thus the blastopore is divided into a dorsal and a ventral half; the latter, called by Balfour the "yolk-blastopore," corresponds to a part of the blastopore of the frog. The dorsal blastopore becomes closed by the medullary folds and gives rise to the neurenteric canal, but the second Elasmobranch peculiarity is that before this closing takes place, the growth of the tail region commences and the hinder end of the embryo presents an open groove leading from the anal region round to the neural tube. It is this phenomenon which has given rise to the idea that the

neural plate is progressively formed by the closing of a slit-like blastopore—an idea entirely negated by the examination of those more primitive developmental histories which we have already described.

Before passing on to consider the development of the Amniota there is one case which demands special attention, because a great deal of weight has been laid on it. That is the description of the development of the Gymnophionan genus *Hypogeophis* by Brauer (8). Here there is a comparatively large yolky egg with a germ-disc which alone segments, though nuclei are found scattered throughout the unsegmented yolk. The superficial layer of the germ-disc becomes differentiated as a well-marked columnar epithelium from the underlying rounded yolky cells. At one point a raised rim makes its appearance, and here according to Brauer an inflexion of the columnar epithelium takes place, leading to the formation of a sac whose roof is columnar epithelium, whilst its floor is composed of rounded yolky cells. At the same time irregular spaces interpreted as a "segmentation cavity" have appeared within the yolky cells themselves. In the next stage the cavity formed by invagination has broken through into the "segmentation cavity," and so a quite roomy archenteron is formed. In the meantime from the opposite site of the germ-disc the ectoderm has swept completely round the egg, and so the blastopore is outlined. The dorsal wall of the archenteron consists partly of cylindrical cells and partly of rounded cells, and Brauer maintains that the part composed of cylindrical cells can be distinctly delineated from the part made of rounded cells; the part consisting of cylindrical cells becomes then separated from the rest by the growth under it of the sides of the yolky layer which constitutes the floor, the sides and front part of the roof of the archenteron, and this sheet of columnar cells is entirely used up in forming the lateral sheets of mesoderm and the notochord, and in this way the epithelium of the alimentary canal comes to be composed entirely of "vegetative" or lower layer cells. Founding on

these facts, it has been inferred that the columnar epithelium of the invaginated sac is ectoderm, and even when this sac has opened into the "segmentation" cavity it is inferred that a sharp line can be drawn between the two kinds of cells. Now with reference to this development there are several points to be noted. First the description labours under the disadvantage of being founded on a limited number of specimens. Brauer found great difficulty in getting his material, and his specimens of young stages were few. In particular a gap occurs about the time of the "breaking through" of the invaginate archenteron, and he has to admit that it is difficult to fix the precise spot where one sort of cell leaves off and the other begins. There is no proof that the place where the "undergrowth" begins corresponds to this point. Next the invaginate cells are different from the ectoderm in several points, and in any case a description of a process founded on scanty specimens in which considerable gaps have to be bridged cannot hold against one like that of Brachet of Siredon founded on an enormous mass of material. The whole thing can be interpreted more simply as follows: Brauer admits that the dorsal lip grows backwards; it is therefore to be regarded as a growing point from which new cells are added to the ectoderm above and to the endoderm below. But we saw that in other Amphibia, before any invagination had taken place or a dorsal lip had grown out, the archenteric cavity appeared as a cleavage amongst the large cells at the lower pole of the egg. Now if we assume that this cleavage is represented in *Hypogeophis* by the so-called segmentation cavity (*s'*), which, however, happens to form, not at the surface of the egg, but further in, and that the "invagination" is the formation of new endoderm from the growing point in the dorsal lip, we shall be able to completely reconcile the development of *Hypogeophis* with that of *Amphioxus* and of *Siredon*. Indeed, in the Sturgeon's egg, as figured by Deane and reproduced in text-figs. 5, 7 and 8, the archenteric cavity is almost divided into two parts, such as I have described. The complete separation

TEXT-FIG. 8.



8.

Median sagittal sections through portions of the eggs of Sturgeon (after Deane), Hypogeophis (after Brauer), Tropidonotus (after Bellowitz), in order to show the process of gastrulation. *s*. The segmentation cavity. *s'*. Inner portion of archenteron. *a*. Outer portion of archenteron.

of the lumen into two parts is of no more importance than the solidity of the œsophagus in many Vertebrate embryos.

The development of the lower Amniota has been worked out in several forms in great detail. The work of Will on the European Gecko *Platydictylus* (36), of Mitsukuri on the turtle *Chelone* (29), and by Ballowitz on the snake *Tropidonotus* (4) have given very concordant results. In all three cases a dorsal lip of the blastopore is formed, not at the extreme edge of the germ-disc, but inside it, and we are apparently justified in assuming that the early growth of the ectoderm from the opposite and anterior side of the germ-disc, which corresponds to the point *y* in the gastrula of *Amphioxus*, and its short-circuiting round a small circle of the sphere represented by the whole egg, has taken place far earlier than it does in the case of the Elasmobranch. If this be granted, the subsequent development bears a strong resemblance to that of *Hypogeophis*. In all three cases a sac-like cavity forms by invagination beneath the blastoporal lip (*a*, text-fig. 8). Another cavity forms by the separation from the yolk of the endoderm cells, which have differentiated themselves from the lowest layer in the germinal disc (*s'*, text-fig. 8). This space, corresponding to the so-called segmentation cavity of *Hypogeophis*, breaks through into the invaginated sac; so an archenteron is formed, the roof of which is formed of the invaginated cells, the floor of yolk, and the sides and anterior slope of "secondary endoderm." After the floor of the invaginated sac and the roof of the secondary endoderm cavity have met and undergone absorption, three parallel folds are formed in the roof of the compound cavity. The centre one gives rise to the notochord, the two lateral to the trunk cœlom on each side. In this way the roof of the archenteron is used up, and the sides of the compound archenteron unite beneath it in the middle line to constitute the roof of the definitive gut. In the higher Amniota, such as *Aves*, the invaginated sac is replaced by an almost or quite solid in-growth of cells, and the dorsal lip of the blastopore becomes drawn out into a slit-like form, the well-known

primitive streak.¹ The division of the solid in-growth into central notochord and lateral mesoderm masses corresponds to the system of grooves found in the roof of the archenteron of lower Amniota.

When we pass to Mammalia, we find that the exact sequence of events in the earliest stages of segmentation is most imperfectly known. The egg is described as forming a solid mass of cells or morula, in one side of which vacuoles appear, which lead eventually to the formation of a space filled with fluid. When the egg reaches the uterus it is in the form of a vesicle with a wall consisting of a single layer (Raubert's layer), attached to one side of which is a mass of cells, called by Hubrecht (18) the embryonic button. From the superficial layer of the embryonic button the ectoderm of the embryo is differentiated, and Raubert's layer over the region of the button disappears. The development of the embryo, once this has taken place, closely resembles that of the chick, so that the principal new question presented for our consideration is the meaning of Raubert's layer. I suggest that it is nothing else than the sheet of ectoderm which in the eggs of Amphibia sweeps round the ventral surface of the egg, and eventually forms the ventral lip of the blastopore. Owing to the loss of yolk by the mammalian egg it is absolutely necessary for its survival that at the earliest possible moment it should be provided with a covering of cells capable of assimilating nourishment from the womb-wall. Hence the normal spreading of ectoderm, i. e. growth of the ventral and posterior lip of the blastopore, takes place long before there is a trace of the dorsal lip, and hence there is differentiated a special layer of ectoderm cells for this purpose even over the embryonic area. That this is a secondary phenomenon is shown by Hill's observation that in Marsupials this is not the case, but that here the embryonic

¹ Wilson and Hill (38) describe in *Ornithorhynchus* a very anomalous condition of affairs, viz. the co-existence of an open blastopore and of a primitive streak some distance behind it.

button is exposed (16).¹ In those forms of mammals in which the embryonic area is invaginated into the vesicle, Rauber's layer does not extend over the embryonic area, but only up to the edges of the invagination. These edges are thickened masses which cohere and form a great plug of ectoderm called the "Träger."

The formation of the coelom in *Amphioxus* as five outgrowths from the gut, viz. a median and two paired outgrowths, is not without parallels amongst other Vertebrata, even in the present state of our knowledge, and it is more than probable that if attention were once directed to this point, a method of development fundamentally similar to that of *Amphioxus* would be found to range throughout the whole of the Vertebrata. In the embryos of the shark *Acanthias* there is found a bilobed outgrowth from the anterior end of the gut, which becomes separated off as a single pre-mandibular cavity, which divides into two cavities, from whose walls the inferior oblique muscle of the eye and the superior inferior and internal rectus muscles are developed. In the mandibular arch lies the so-called mandibular cavity, the origin of which has not been traced. From its upper extremity, which extends forwards over the pre-mandibular cavity just as the collar-cavity of *Amphioxus* extends over the head-cavities, the superior oblique muscle is developed, whilst from its lower portion constrictor muscles of the throat are developed. A pre-mandibular cavity originating from the gut has been described in the lizard, and by Edgeworth in the chick (14). Pre-mandibular and mandibular cavities have been described in Urodela, Elasmobranchii, and in all Amniota in which they have been looked for; in Anura and Dipnoi they are apparently represented by solid masses of cells, as Agar has pointed out (1). That the trunk-cavities

¹ I had the pleasure of listening to Professor Hill's exposition of this point and of seeing photographs of his preparations at the Dublin meeting of the British Association in 1908. On referring to the 'Proceedings' of the Association I regret to find that this interesting paper is reported by title only.

are represented by the great solid sheets of mesoderm cut out from the sides of the archenteric roof termed the "mesodermic bands," requires no special demonstration.

To sum up, the differences between the development of *Amphioxus* and the development of the higher *Vertebrata* can be explained on the simple assumption that there has been a progressive increase in food-yolk, and that this yolk for the most part has been stored in the ventral wall of the archenteron, which has been thereby rendered relatively inert. This has led to a modification of the process of invagination, which retains its primitive features in connection with the dorsal lip of the blastopore, but ventrally is changed to a process of slipping over or epibole. At the same time the processes of folding which give rise to the *cœlom* in *Amphioxus* become modified so as to give place to the outgrowing of solid masses of cells.

Turning now to Professor Hubrecht's account (18) of the ontogenetic processes in *Vertebrata*, we find that he entirely reverses the method which I have followed. Instead of explaining the more complex development of the higher forms as a modification of that of the simpler forms, he takes the development of mammals as a starting-point, and then proceeds to read into the development of the lower forms what he finds there. Since in the mammalian egg the cells destined to form notochord and mesoderm arise by invagination, therefore they are ectoderm and for them the name "protochordal wedge" is given. It does not, however, escape Hubrecht that these invaginated cells come into continuity in front with cells which he regards as true endoderm, and therefore in front of the protochordal plate there is an endodermic protochordal plate, and the notochord, if I understand him aright, arises from both, and is therefore a compound structure. Further, Hubrecht is a convinced believer in the formation of the neural plate by the gradual closing of a long slit-like blastopore. This process he dignifies with the name "notogenesis." To read a complex process like this into the development of *Amphioxus* appears to me a sheer impossibility.

In support of it, it is true, Hubrecht figures from Legros an oblique longitudinal section of an abnormal *Amphioxus* gastrula, which is utterly unlike the appearance presented by any normal embryo. Legros admits the section to be oblique, a fact of which Hubrecht does not apprise his readers. Hubrecht regards Rauber's layer as a special larval envelope and utterly distinct from the true ectoderm. He imagines that the aquatic ancestor of Mammalia had a larva in which there was such an envelope which was afterwards cast off, and cites certain Trochophore larvæ as analogous instances. When the aquatic ancestor took to a land life the free-swimming larva was retained within the womb of the mother, and so the peculiar development of Mammals was attained. According to this reasoning, then either birds and reptiles arose from a different stock from Mammals, or else the oviparous method of development which they exhibit was secondarily developed out of a previous viviparous condition. Now on this view several remarks may be made. Rauber's layer is not analogous to the investing layer of the *Sipunculus* larvæ as Hubrecht imagines, because in the latter case we have to do with a median belt of larval ectoderm which develops into a broad ciliated band overlapping the remaining ectoderm before and behind. The loss of this belt in the Trochophore larva leaves a wound which is closed by the cicatricial union of the ectoderm produced by the head and tail blastema respectively, whereas Rauber's layer is an outer layer of ectoderm according to Hubrecht. Then, whichever alternative we take of the ancestry of Mammalia, we are beset with difficulties. To maintain that they are the offspring of a distinct stock from that which gave rise to birds and reptiles is a supposition which may be left to the tender mercies of comparative anatomists, who will make short work of it. Every recent discovery in palæontology tells against such a supposition; the mammalian vertebral column is constructed on the reptilian plan, whilst the amphibian one is built on a different plan, and so on. But if we admit the existence of a common ancestral stock of birds, reptiles, and

mammals, then the change from a viviparous to an oviparous method of development, which Hubrecht must postulate, is totally unthinkable. How should an animal which had once adopted the habit of carrying the young in the womb—the safest method of development, and the one which rendered the parent completely free from the necessity of visiting any fixed place for parturition—revert to the dangerous and primitive method of laying eggs? In every other case in which viviparity occurs in the animal kingdom we have evidence that it has developed out of oviparity, not vice versâ. Hubrecht lays great stress on supposed indications of a specially differentiated layer of ectoderm over the embryonic areas of the embryos of reptiles, like *Sphenodon* and of *Echnida*, as a proof of their descent from viviparous forms. Hill expressly denies the existence of such a layer in *Ornithorhynchus* (38) and the marsupials (16), but even if the facts were as Hubrecht represents them it by no means proves his case. The ectoderm in all the higher Vertebrata is typically many-layered, and that an outer layer should prematurely become differentiated is only an anticipation of adult conditions. Even in Elasmobranchii and Cyclostomata, which Hubrecht classes together with *Amphioxus*, and separates from all other Vertebrata on account of their single-layered embryonic ectoderm, eventually develop a many-layered adult ectoderm. Natural selection may have seized on this tendency when Rauber's layer was evolved.

The way I have suggested of looking at the evolution of Vertebrata, which has the audacity in these days of innovation to be commonplace, escapes all these difficulties. All grades in the development of viviparity are met with amongst living reptiles, and it does not require a very violent exercise of the imagination to pass from a condition such as is found in *Zootoca* to that found in *Ornithorhynchus*, for example.

But Hubrecht claims for his view that it enables him to explain the origin of the allantois and the amnion as embryonic envelopes in a satisfactory way. He pictures the

process as far as I can follow him in some such manner as this: Starting with a supposed holoblastic egg which had a larval envelope, the mass of cells destined to form the embryo does not develop with equal rapidity to the surrounding envelope, and consequently becomes detached from it everywhere except at one spot, where there is a stalk of connection between envelope and embryo. The amniotic cavity between true amnion and embryo was originally a water cushion developing within the ectoderm to form a protection for the embryo, which subsequently derived a more complete protection by the method of detachment just described. Along the stalk of connection between embryo and vesicle the bladder subsequently grew, and so the allantois was formed. From this process the simple folding process seen in the formation of the amnion in Reptilia is supposed to have arisen as a secondary modification. Now an amnion has been developed in Insecta, and Hubrecht will find it hard to convince any specialist in Arthropoda that it has come about otherwise than as a modification of the folding of the germ-disc seen in its incipient stages in Myriapoda. But why, if a protective fold has developed in the Arthropodan egg, should there be any difficulty about its development in the Vertebrate egg? Suppose we apply in both cases the same hypothesis. The folding of the germ-disc in a Myriapod egg is due to its great length and the consequent impossibility of its expansion within the egg-shell. In the insect egg the germ-disc is proportionately shorter, but the inherited habit of folding has persisted and has led to the formation of the amniotic fold.

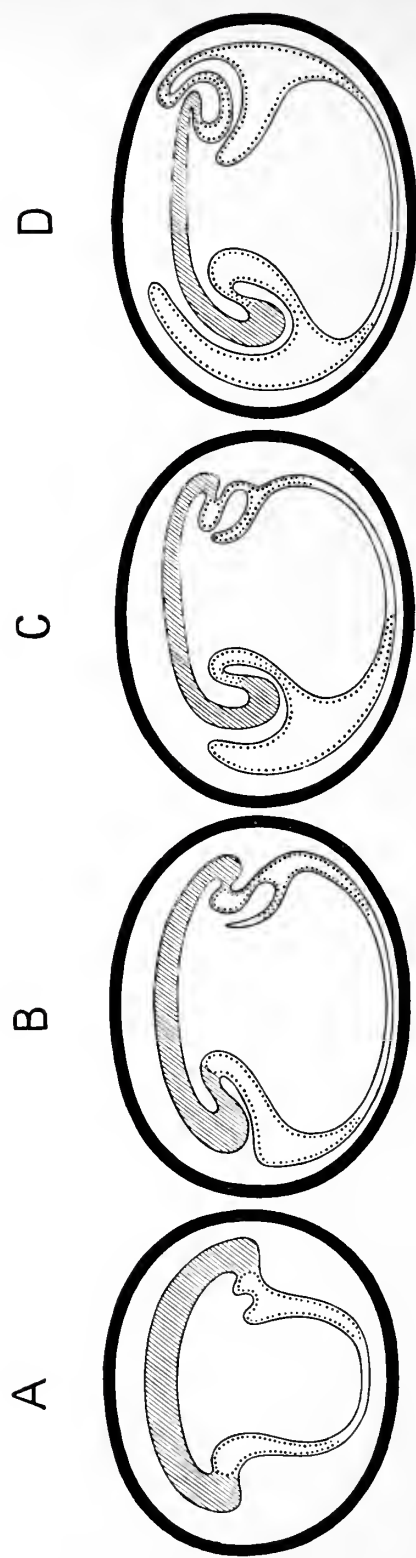
Now in the Amphibian egg no expansion of the embryo takes place till it has escaped from the egg membrane, but when the Reptilian ancestor took more completely to life on land a longer retention of the embryo within the egg-shell and a greater supply of yolk would become a necessity. Hence at a certain stage in the development of land animals there arose a necessity of bending the head inwards into the yolk-sac so as to give room. The most flexible part of the

yolk-sac was the so-called pro-amnion, which lay in front of and beneath the head, and here the flexure took place. The mere fact that in Mammalia there is here no meeting of the lateral sheets of mesoderm, so that the diploblastic head envelope is called "pro-amnion," whereas in reptiles the lateral sheets of mesoderm early meet beneath the head, and so a "true head fold of the amnion" is developed, seems to me to be a secondary affair. This bending tended, just as a ship sinking by the head tends to lift her stern, to raise the tail region and so bring the bladder-like outgrowth of the gut—already present in Amphibia—near the surface of the egg, and so to increase its chances of getting oxygen. In this the foundation was laid for the modification of the allantois into a breathing organ and for the corresponding development of the tail-fold of the amnion, the two developing, as Balfour long ago showed, together, since the tail-fold of the amnion contains the extension of the bladder or allantois.

Hubrecht scornfully asks if the pro-amnion was developed to contain the head, why there is none in the human embryo? The answer is easy; in the human embryo the embryo itself is as long as the yolk-sac from the beginning, the yolk-sac being in this case a vestigial organ which has suffered great reduction in size, and hence when the embryo elongates there is no yolk-sac for it to plunge its head into. Space, however, will not permit us to pursue this subject further. To an author like Hubrecht, who finds no difficulty in supposing that the oviparous mode of development in Echinida is secondarily derived from a placental method of development, such as is found in the rabbit, no change is so unlikely as to seem impossible.

Apart from Hubrecht's desire to prove that the ancestors of Mammals never had yolky eggs, the main result of his paper is to advocate the view that Vertebrates have developed from an Actinian ancestor. The protochordal wedge represents, according to him, the old stomodæum which opened into the true endodermal gut below. The old mouth was originally surrounded by a nerve ring, but it became closed

TEXT-FIG. 9.



9.

Stages in the evolution of the amnion and allantois. The thick black line is the egg-shell. The cross-barred region the body of the embryo. The colonic epithelium is represented by a dotted line. The gut and its outgrowths the yolk-sac and the bladder are left white.

from in front backwards and the anus is the only remnant of it; the present mouth is a new formation. In this theory Hubrecht supposes that he is reviving an old theory of Sedgwick's as to the origin of the Metazoa (30). But on Hubrecht's theory the cœlom is of ectodermal origin, and must have originated from stomodæal pockets which do not exist in Actinozoa, whilst Sedgwick regards the cœlom as derived from the inter-mesenteric spaces of the true endodermal gut. Sedgwick's theory was published about a quarter of a century ago, and was based on a review of all the evidence available at the time. It is a theory of the origin not of Vertebrata but of all Metazoa, and it traces them back to an Actinia-like ancestor. The theory of Sedgwick may be analysed into three parts—for the name Actinia given to the common ancestor was, of course, only an indication of a very general resemblance to a modern sea-anemone. The three parts are: (1) The position that the cœlom arose as pockets of the original gut or archenteron; (2) the position that mouth and anus are two separated portions of a long slit-like mouth; and (3) the idea that the central nervous system is homologous throughout the whole of the Metazoa and had originally the form of a ring round the original mouth. Now I venture to maintain that the first two parts of the theory have received more and more support as embryological research has gone on, but that the third part must be given up and that the common Metazoan ancestor in consequence takes on more resemblance to a Ctenophore than to an Actinian, for it has become evident that in many types of larvæ the apical plate of neuro-epithelial cells is the first rudiment of the brain, and that it is independent of post-oral nervous aggregations. That the cœlom arises in *Amphioxus* as five archenteric outgrowths I trust I have convinced the readers of this paper. That the mouth and anus in all animals in which two such openings are found, owe their origin to the division of a long slit-like cœlenterate mouth I firmly believe. If, however, the affinities of *Amphioxus* are to be sought for in the vicinity of *Balanoglossus*, then

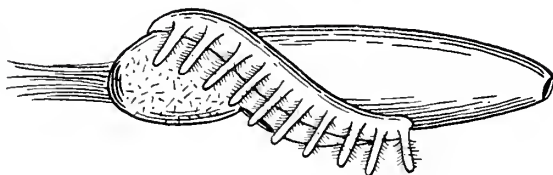
an excellent reason can be given why we cannot expect to find a trace of the slit-like mouth in Vertebrate ontogeny, for there is no trace of it in the development of *Balanoglossus* nor in the development of the Echinodermata, which is the group most nearly allied to *Balanoglossus* and the Enteropneusta. In both Enteropneusta and Echinodermata the blastopore becomes the anus and the mouth is formed later as an independent meeting of ectoderm and endoderm. I am strongly inclined to believe that the "seam" which originally connected mouth and anus was situated on the ventral and not on the dorsal surface. It is quite possible that the second stage in gastrulation, viz. the upgrowth of the ventral lip and the coincident union of its lateral halves, may be a reminiscence of this closure. The great difficulty in fixing points of reference in Vertebrate development is that general growth in length supervenes so early that before the mouth is formed the embryo has altered in size and shape to a great extent. Nevertheless the place where the mouth is formed in *Amphioxus* cannot be far from the point *y* in the gastrula in fig. 6, in which the upgrowth of the ventral lip has not yet begun.

Now *Balanoglossus* presents us with a condition where the nervous system, as in some Nemertinea and Echinodermata, is practically co-terminous with the ectoderm—a condition therefore in which a central nervous system could originate anywhere where stimuli were concentrated. The local concentration in the dorsal region of the collar which we believe to be the forerunner of the earliest part of the Vertebrate dorsal nerve-tube receives its explanation from the structure of the other Enteropneusta, *Cephalodiscus* and *Rhabdopleura*. In both of these animals the collar region is produced into a series of arm-like outgrowths beset with ciliated tentacles. The dorsal nerve cord is a centre co-ordinating the two halves of this apparatus. The free-swimming pelagic ancestor of Enteropneusta probably possessed similar developments of the collar region, and it is interesting to reflect that the ciliated "cirri" which fringe

the edge of the oral hood in *Amphioxus* occupy a corresponding position to the "arms" of *Cephalodiscus*, and are in all probability homologous therewith. Adding the "arms" to the collar of a *Balanoglossus* and shortening the worm-like trunk, the length of which is a result of burrowing life, we arrive at a conception of the primitive Vertebrate stock to which the free-swimming ancestors of Echinodermata were allied. In the Echinodermata the fixed habit was adopted and one of the "collar-cavities" grew at the expense of the other and so the water-vascular ring was formed.

Professor Hubrecht indicates a belief that the "supposed Actinian ancestor developed into a vermiform one." What

TEXT-FIG. 10.



10.

The common ancestor of Vertebrata, Enteropneusta and Echinodermata.

kind of a "vermiform" ancestor Hubrecht means he does not indicate in the paper under discussion. To judge from his previous work one would suppose that the "worm" was a Nemertean. The theory of the Nemertean origin of Vertebrata was published in this Journal in two editions. In the later and revised edition (17) we learn that the Vertebrate nervous system corresponds to an inconspicuous dorsal nerve in the Nemertinea, whilst the main nervous system of those "worms" gives rise to the chain of cranial ganglia. The proboscis of Nemertinea becomes the hypophysis of Vertebrata, whilst the proboscis-sheath forms the notochord. The question of the coelom is passed over as a difficult and obscure question. As, however, the figure he gives of the vermiform ancestor in the present paper is adorned with a number of

cœlomic pockets, one is tempted to believe that he means to give his adhesion to the famous "Annelidan" theory of the origin of Vertebrates put forward by Dohrn, to which we owe such a quantity of excellent work. This seems the more likely, as in the twenty-two years which have elapsed since its final revision the Nemertinean theory has gained little or no support. That the Nemertinea are distantly related to the common stock of Echinodermata and Vertebrata is quite possible; but that they stand anywhere near the direct line of descent is negatived by the consideration that the Pilidium larva in its development stands near the Trochophore larva of Annelida, and is widely different from the Tornaria larva of Balanoglossus or the allied Dipleurula larva of Echinodermata. The Annelidan theory did justice to a number of remarkable resemblances between Annelida and Vertebrata. In proportion, however, as zoological research has advanced, it has transpired that most of these resemblances—cf. ciliated tubes leading from the body-cavity to the exterior, development of genital organs from the lining of the body-cavity, etc.—are common to a wide range of cœlomate animals, and only one specific resemblance is left, viz. the metameric repetition of organs, and above all the metameric segmentation of the muscles in the two groups of animals. On the other hand, if Vertebrata were derived from Annelida, we are forced to assume the production of a new mouth and the abandonment of the old one, and a total change in the manner of developing the nervous system, whilst gill-slits and notochord must have been developed entirely *de novo*.

The great advantage of Bateson's theory of the origin of Vertebrata is that we are not forced to make any such violent reconstruction as the formation of a new mouth, and of notochord and nerve-cord we already have the beginnings. All we require to postulate is the appearance of metamerism, and surely this is no unreasonable assumption considering how often the repetition of similar organs crops up in the most widely separated phyla of the animal kingdom.

Of course Hubrecht may reply that there is no *à priori* impossibility in the formation of a new mouth. There is certainly no analogy for it, and if it is legitimate morphological reasoning to assume such changes of function as are implied in it, no valid objection can be brought against the attempts to evoke a Vertebrate from an Arachnid like *Limulus*, in which Patten and Gaskell display such diabolically brilliant ingenuity.

The general conclusion of our study may be summed up thus:

The process of gastrulation in *Amphioxus* leads to the formation of a single layer of invaginated cells, which there is no valid reason for analysing into two kinds.

The closure of the blastopore in *Amphioxus* is due to the concrescence of the lateral lips of the blastopore and to the upgrowth of the ventral one. By these processes the ventral, not the dorsal surface of the embryo is formed.

The mesoderm owes its origin to the outgrowth of five cœlomic pouches from the archenteron in the same manner as the cœlom of *Balanoglossus*.

The formation of the layers in other Vertebrata can be derived from that of *Amphioxus* by allowing for first the disturbing influence of the accumulation of food-yolk in the ventral wall of the archenteron, and, secondly, in Mammalia the disturbing effect of contact with the maternal uterus. In starting with Mammalia, and reading their complicated processes into the development of lower Vertebrata, Professor Hubrecht has read the book of Vertebrate development upside down.

McGILL UNIVERSITY,

April, 1909.

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EXPLANATION OF PLATES 18—21,

Illustrating Prof. E. W. MacBride's paper on “The Formation of Layers in *Amphioxus*.”

[All the figures in this paper were drawn under the magnification afforded by a Zeiss apochromatic immersion objective of 2 mm. focal distance. The original magnification of 900 to 1000 diameters has been retained in the case of the smaller figures, but the magnification has been reduced in the case of the larger to 600 diameters for convenience in reproduction.]

LIST OF ABBREVIATIONS.

a. Anal diverticulum. *a'*. Upper section of archenteron. *at.* Rudiment of atrial ridge. *br.ect.* Ectodermic portion of gill-pouch. *br.end.* Endodermic portion of gill-pouch. *ch.* Notochord. *club.* Club-shaped gland. *coll.* Collar-cavities. *f.* Ectodermic folds which extend over the nerve-plate. *k.* Karyokinesis (mitosis). *l.coll.* Left collar-cavity. *lh.* Left head-cavity. *l.spl.* Left splanchnoceles. *m.* Place where the mouth will be formed. *musc.* Muscular fibres. *my.* Myocœle. *n.c.* Neural canal. *n.e.* Neurenteric canal. *neph.* Hatschek's nephridium. *oc.* Clear cells; first indication of eye-spot. *r.coll.* Right collar-cavity. *r.h.* Right head-cavity. *r.spl.* Right splanchnoceles. *tr.* Trunk-cavity. *v.* Vacuole in spot where left head-cavity will acquire an opening to the exterior. *x.* Position of centre of growth which initiates gastrulation. *y.* Position of centre of growth which closes the blastopore.

PLATE 18.

All the sections on this plate are magnified 600 diameters.

Fig. 1.—Median sagittal section through a flattened blastula in which the centre of growth at *x* is just making its appearance.

Fig. 2.—Median sagittal section through a slightly older embryo in which gastrulation has just begun.

Fig. 3.—Medial sagittal section through a still older embryo in which gastrulation has proceeded further.

Fig. 4.—Median sagittal section through an embryo in which the difference in staining quality between ectodermal and endodermal nuclei is beginning to appear.

Figs. 5 *a* and *b*.—Two sagittal sections through an embryo in which the nerve-plate (*n.p.*) is just beginning to be recognisable. Fig. 5 *a* is a median section, and in it the rounded character of the cells in the neighbourhood of the growing point *x* is remarkable. Fig. 5 *b* is a more lateral section (four sections to the right) in which the neighbourhood of *x* is composed of quiescent cells.

Fig. 6.—A median section through a gastrula in which the archenteron has become hemispherical.

PLATE 19.

Figs. 7 *a* and *b*.—Two sagittal sections through an embryo in which the process of elongation has begun. Fig. 7 *a* is a median sagittal section and fig. 7 *b* a more lateral section. The long axis of the nerve-plate is at right angles to the diameter of the wide blastopore. Magnification 600 diameters.

Fig. 8.—Median sagittal section through a gastrula in which the blastopore is in process of being closed through the activity of the growth-centre at *y. ch*. The layer of cells which will form the notochord and which is already beginning to lose its yolk-granules. Magnification 600 diameters.

Fig. 9.—Horizontal section through an almost complete gastrula taken near the middle of the series. Magnification 600 diameters.

Fig. 10.—Horizontal section through an almost complete gastrula taken near the upper and anterior lip of the blastopore. Magnification 600 diameters.

Figs. 11 *a-e*.—Five transverse sections through a just complete gastrula in which the first traces of the body-cavities can be seen. In fig. 11 *a*, the most anterior section, the collar-cavities (*coll.*) alone are visible. In fig. 11 *b* both collar-cavities and trunk-cavities are seen. In fig. 11 *c* only trunk-cavities are visible. In fig. 11 *d* the anal diverticulum (*a.*) is seen still connected with the more dorsal part of the archenteron (*a'*.) by a narrow canal. In fig. 11 *e* the anal diverticulum is shut off from the rest of the archenteron. *ne.* Neurenteric canal. Magnification 675 diameters.

PLATE 20.

Figs. 12 *a-d*.—Four transverse sections through a larva in which two somites have been cut off from the trunk-cavity. In fig. 12 *a* the openings of the collar-cavities are seen on both sides. In fig. 12 *b* the collar-cavity is closed on the left side and lies on the outer side of

the closed front end of the trunk-cavity. The collar-cavity is still open to the gut on the right side; in fig. 12 *c* it is closed off on both sides. In fig. 12 *d* the posterior ends of the trunk-cavities are seen to open into the archenteron. Magnification 900 diameters.

Fig. 13.—Transverse section through the front end of a larva in which four somites have been cut off from the front end of the trunk-cavity to show the origin of the head-cavities. *pore*. The neuropore. *l.h.* Left head-cavity. *r.h.* Right head-cavity. Magnification 900 diameters.

Fig. 14.—Transverse section through the front end of a larva in which five somites have been cut off from the front end of the trunk-cavities. The head-cavities are more advanced in development, but still open into the gut. *r.coll.*, *l.coll.* The anterior ends of the right and left collar-cavities respectively. Magnification 900 diameters.

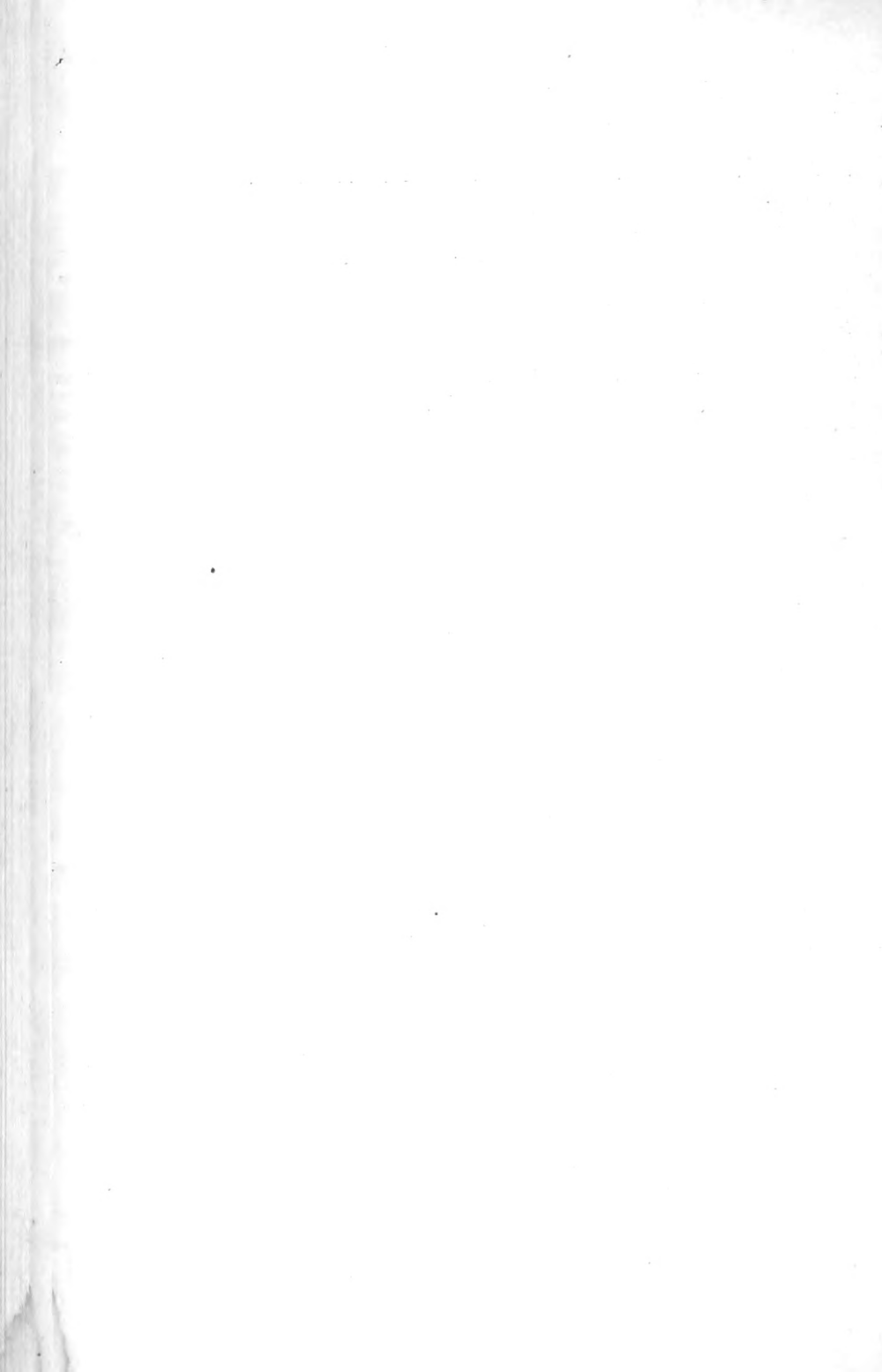
Fig. 15.—Transverse section through the front end of a larva about two days old to show the persistent connection of the left collar-cavity with the pharynx, out of which it appears probable that Hatschek's nephridium (*neph.*) developed. Magnification 1000 diameters.

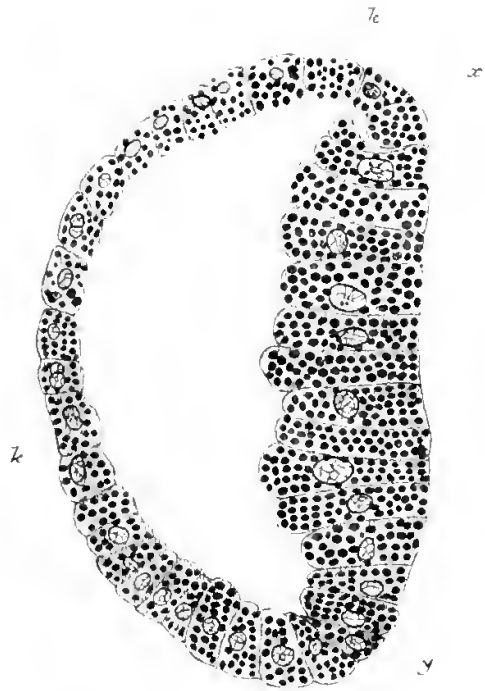
Figs. 16 *a-g*.—Seven transverse sections through a larva of about the same age as that represented in fig. 15 before the mouth is open. In fig. 16 *a* the still open neuropore (*pore*) is seen, and below the notochord the thin-walled right head-cavity. In fig. 16 *b* the left head-cavity is seen completely closed off from the gut, and below it the right head-cavity. At *v.* a vacuole is seen in an ectoderm cell, which marks the spot where this cavity will eventually acquire an opening to the exterior. In fig. 16 *c* the thickening of the ectoderm (*m.*) is seen, which marks the spot where the mouth will break through. The collar-cavities are seen at the sides of the gut extending towards the mid-ventral line. In fig. 16 *d* the club-shaped gland is seen originating as a hollow outgrowth from the wall of the pharynx, and the posterior extensions of the collar-cavities are seen below the splanchnocoel, which have been formed by the fusion of the ventral parts of the posterior somites. *my.* The myocoel of one of the myotomes. *musc.* Muscular fibres on the inner walls of these myotomes. In fig. 16 *e* the first gill-slit is seen to originate by the meeting of an ectodermal in-growth (*br.ect.*) and an endodermal outgrowth (*br.end.*). In fig. 16 *f* the anal diverticulum is seen, and the expansion of the ectodermal cells to form the caudal fin (*fin*), which is seen as a cuticular rim. In fig. 16 *g* the solid seam of cells representing the neurenteric canal is seen. Magnification 1000 diameters.

PLATE 21.

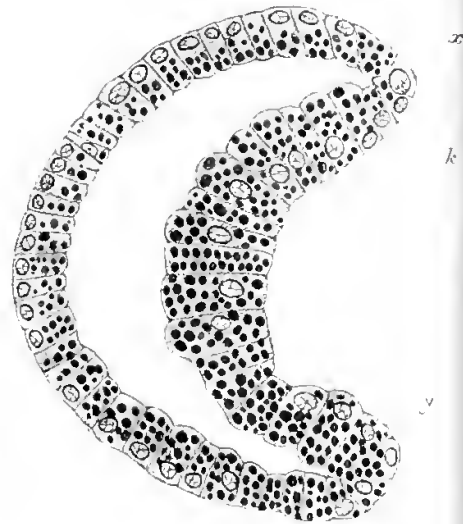
Figs. 17 *a-h*.—Eight transverse sections through a larva in which the mouth and first gill-slit have been formed, and in which the left head-

cavity has acquired an opening to the exterior, slightly older than that represented in fig. 16. In fig. 17 *a* the left head-cavity is seen opening to the exterior. In fig. 17 *b* the collar-cavities are seen extending down at the sides of the pharynx, and the external opening of the club-shaped gland is seen at *club* in front of the mouth. In fig. 17 *c* the mouth is seen, and on the posterior extension of the right collar-cavity (*r.coll.*) an ectodermal thickening (*at.*), the rudiment of the atrial ridge. Figs. 17 *d, e, f, g*, and *h* are five consecutive sections through the hinder end of the pharynx behind the gill-slit, which is grazed in fig. 17 *d* (*br.end.*) to show how the extensions of the collar-cavities thin out and disappear, whilst right and left splanchnoceles meet in the mid-ventral line. Magnification 1000 diameters.

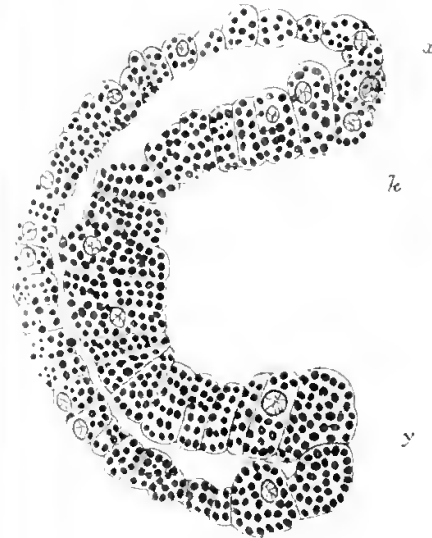




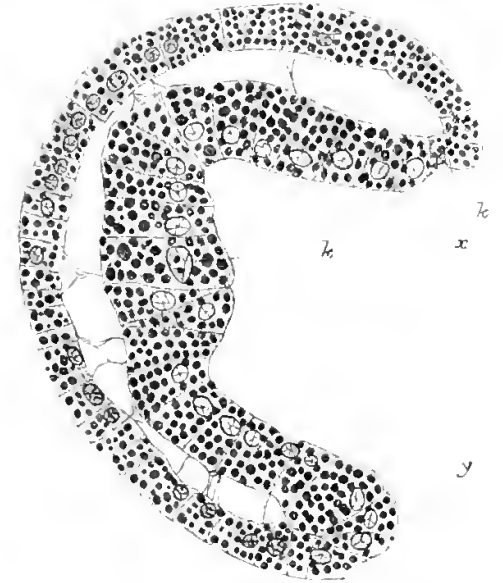
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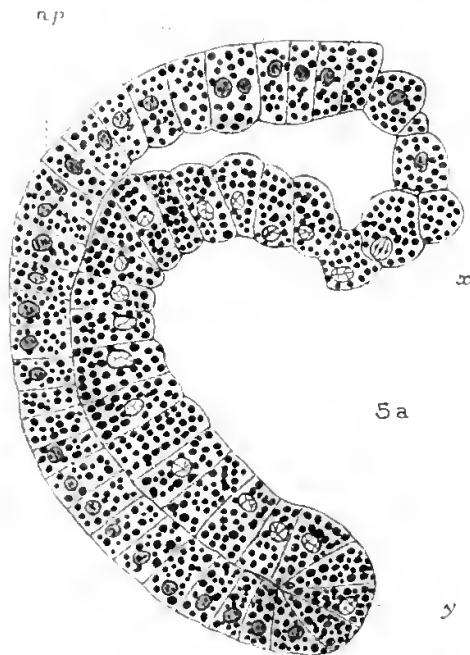
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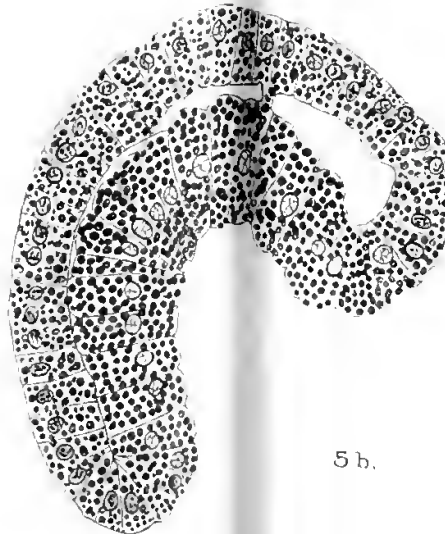
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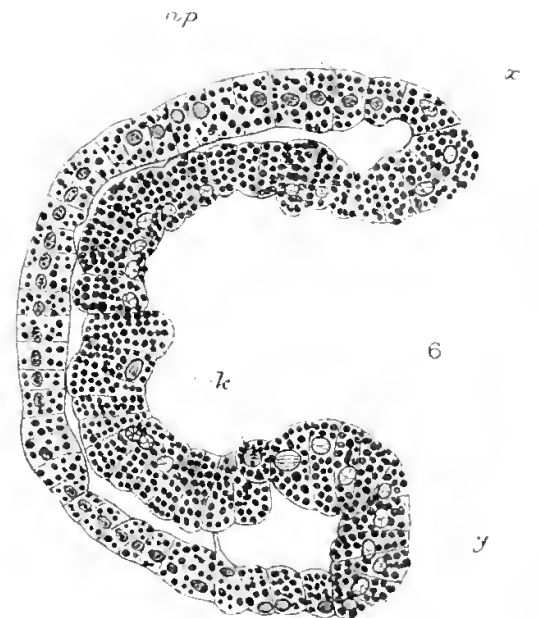
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5a

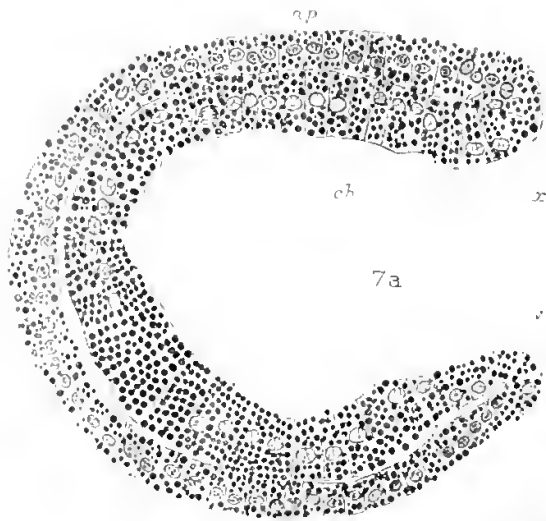


5b.

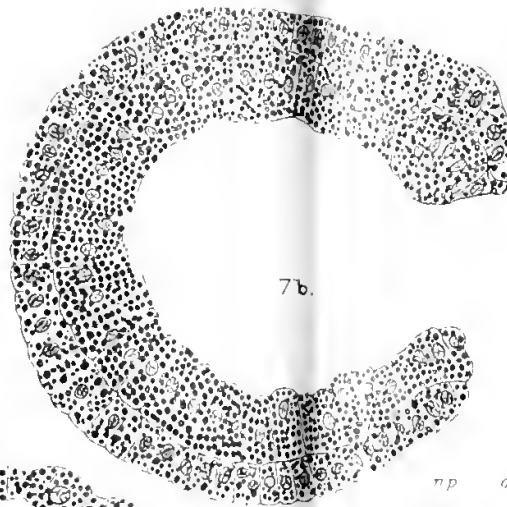


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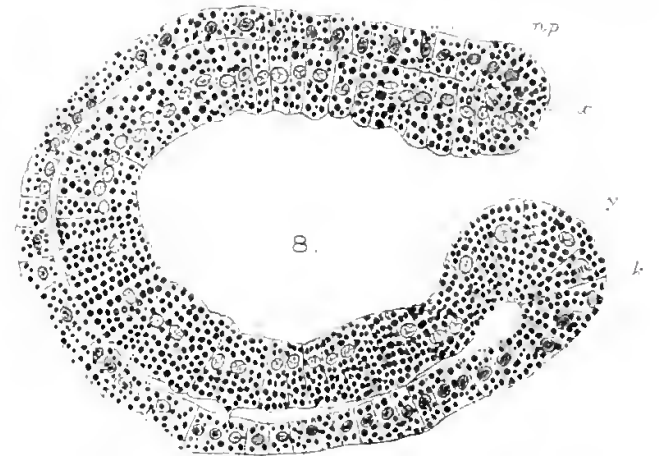




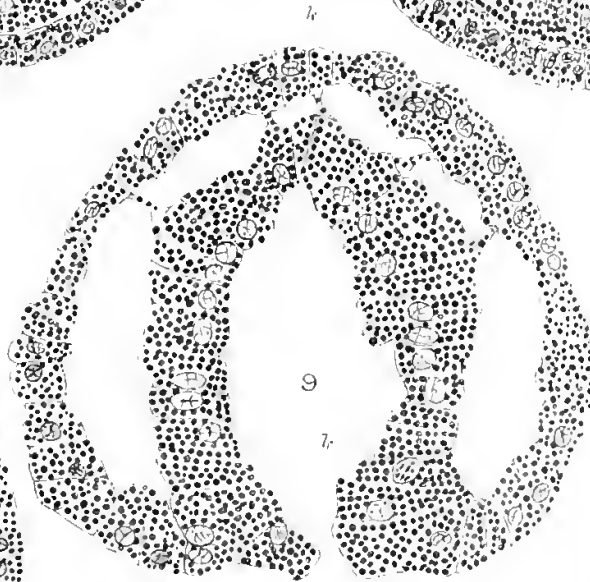
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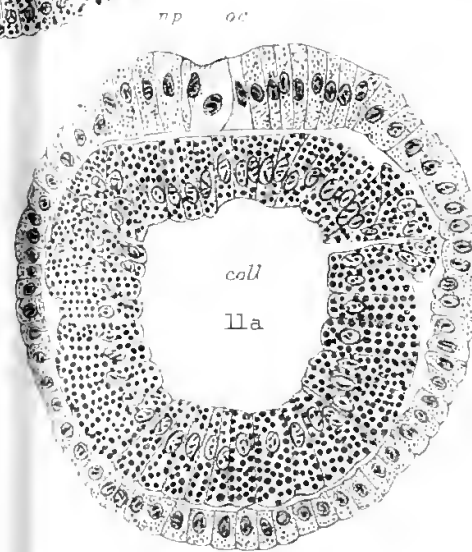
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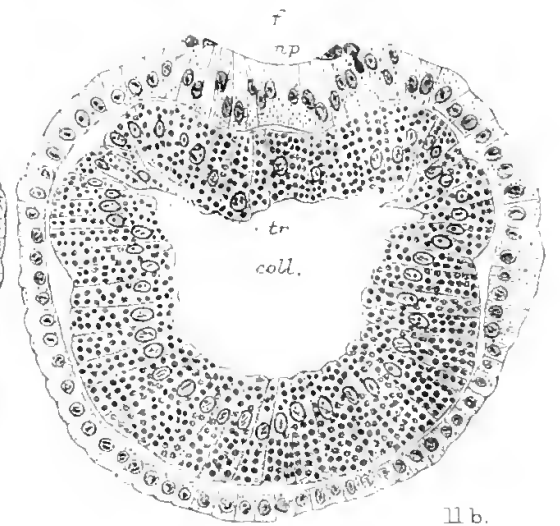


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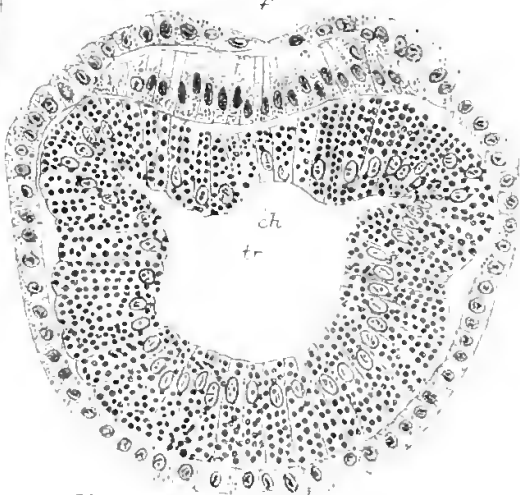
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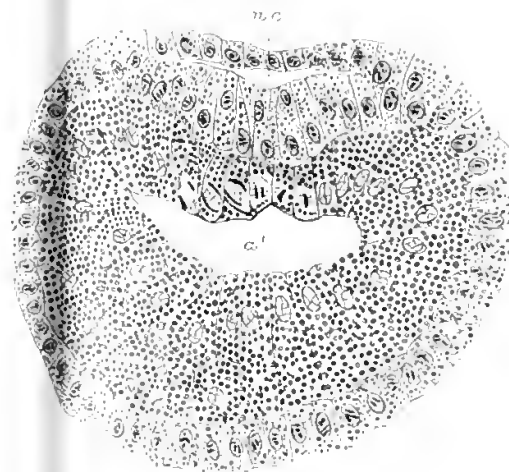
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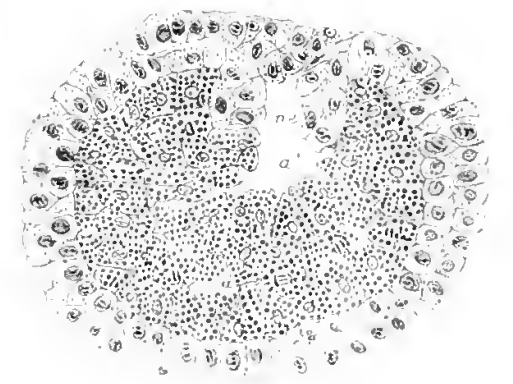
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np

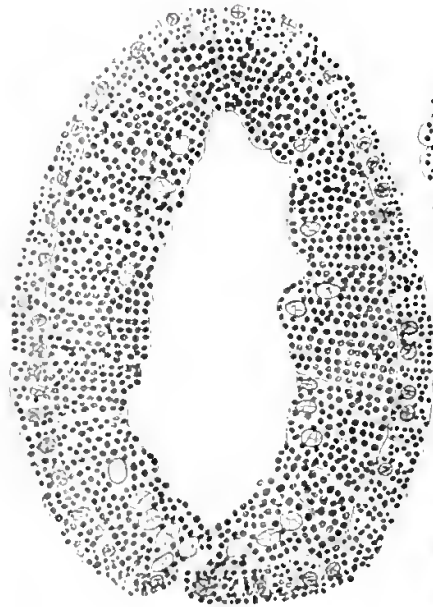
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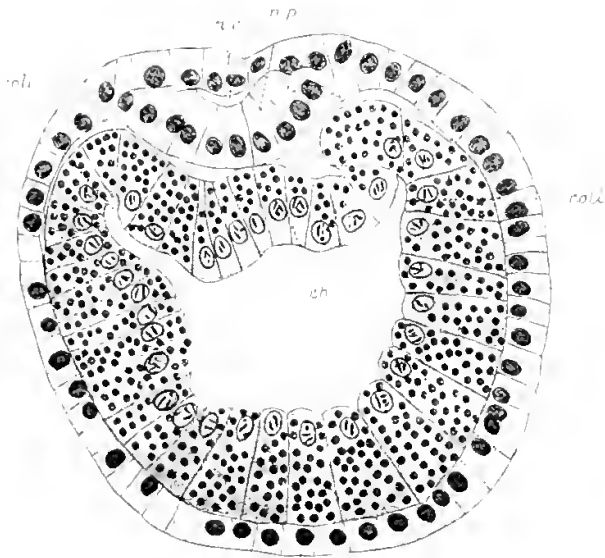
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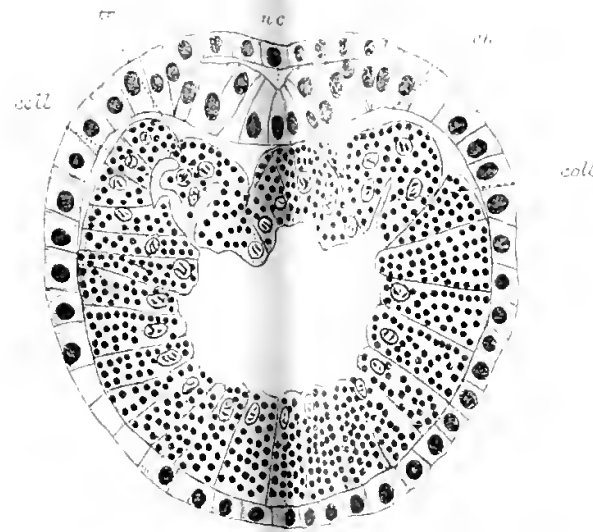
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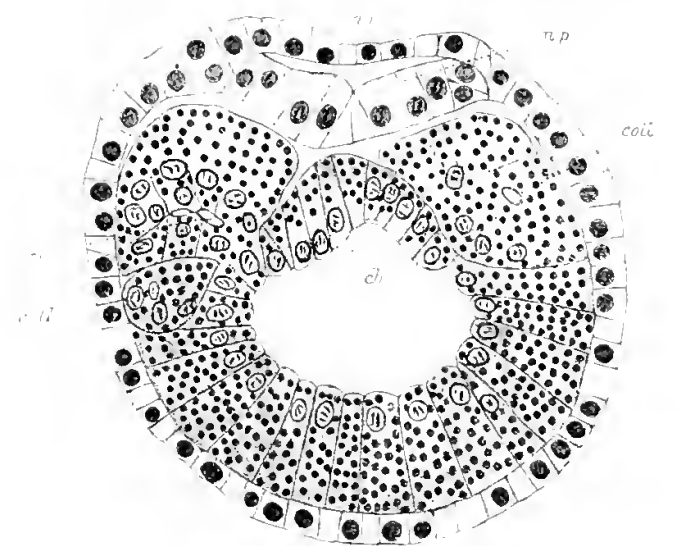




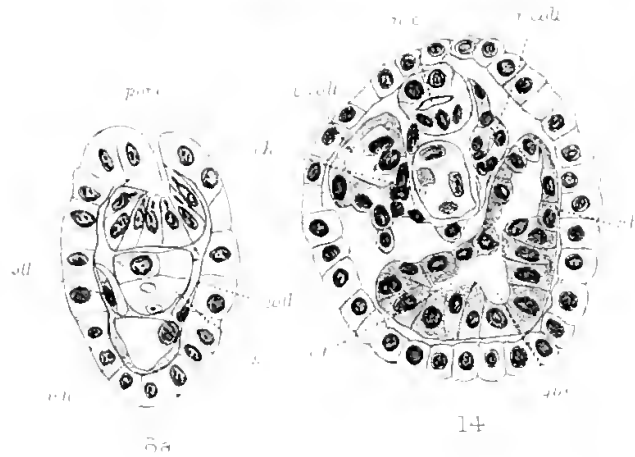
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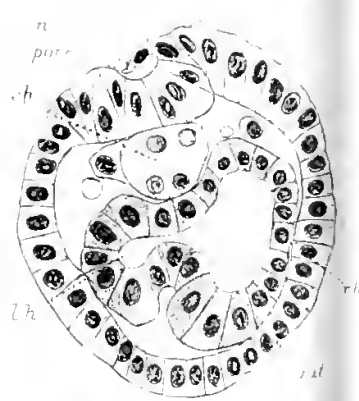
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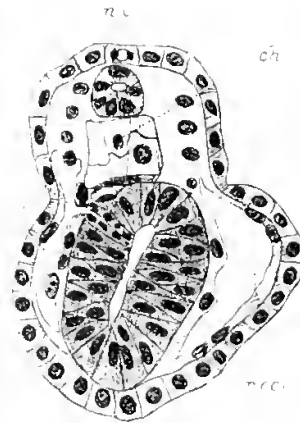
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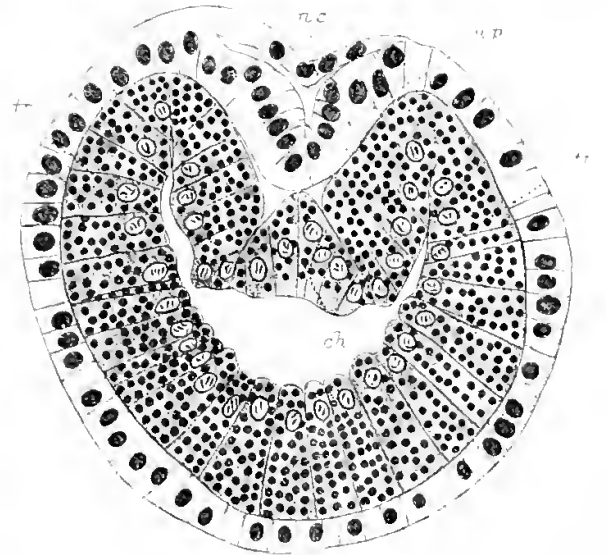
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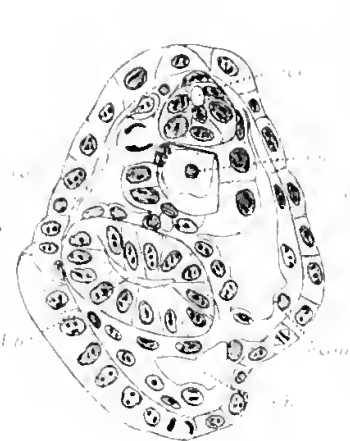
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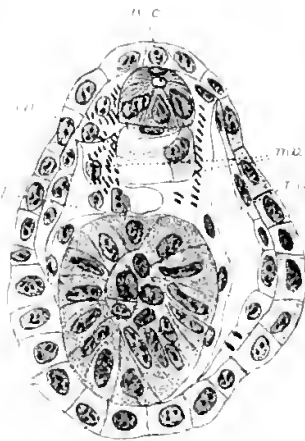
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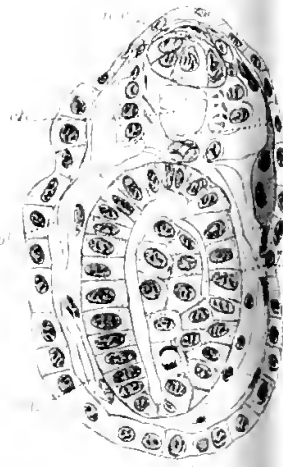
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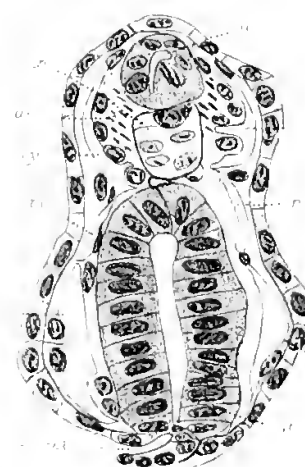
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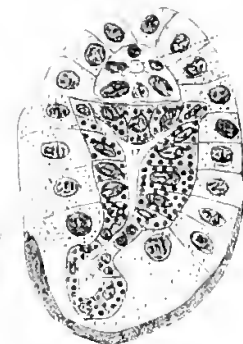
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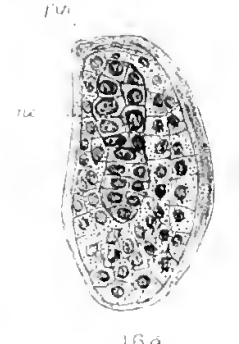
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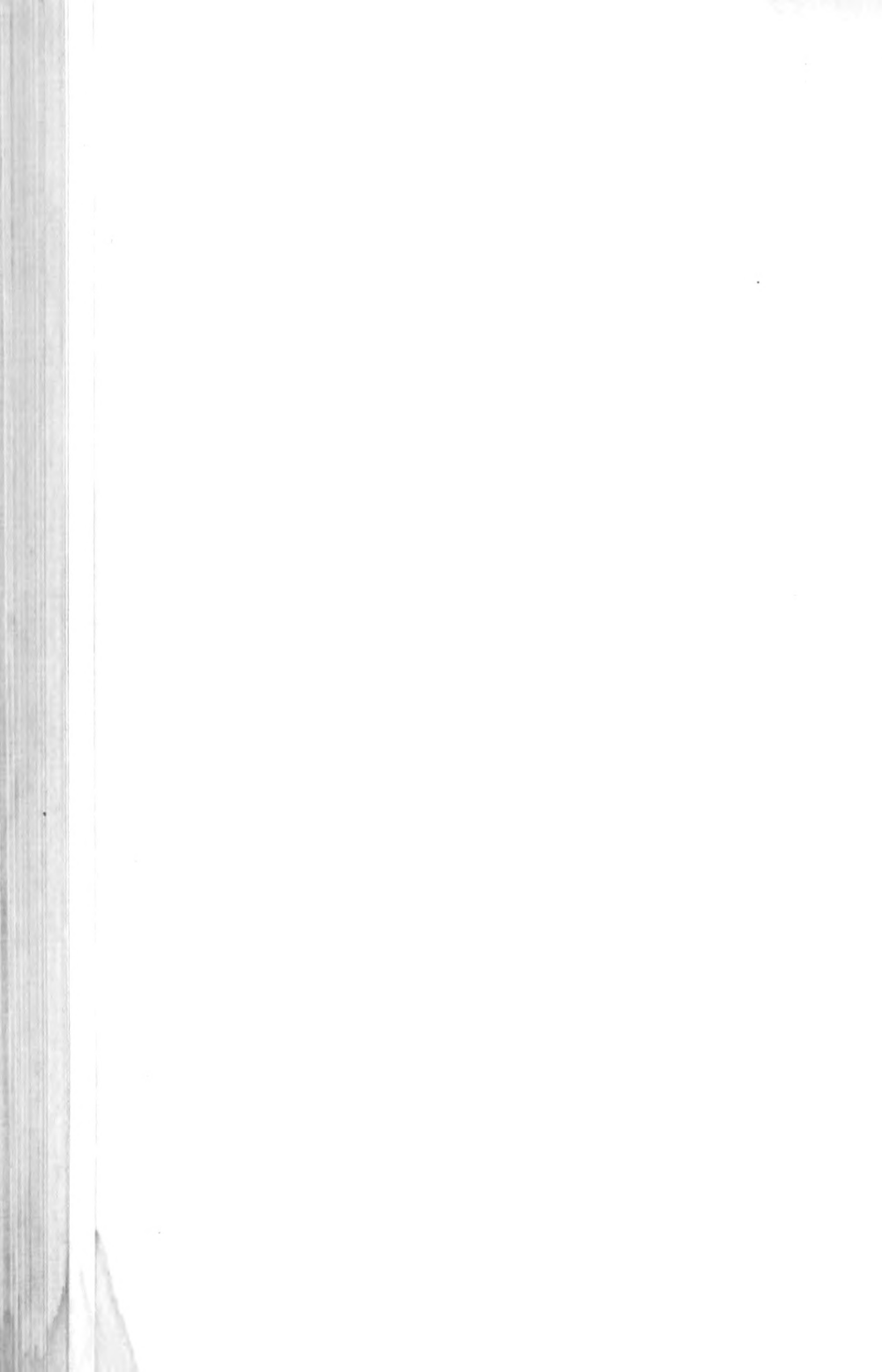
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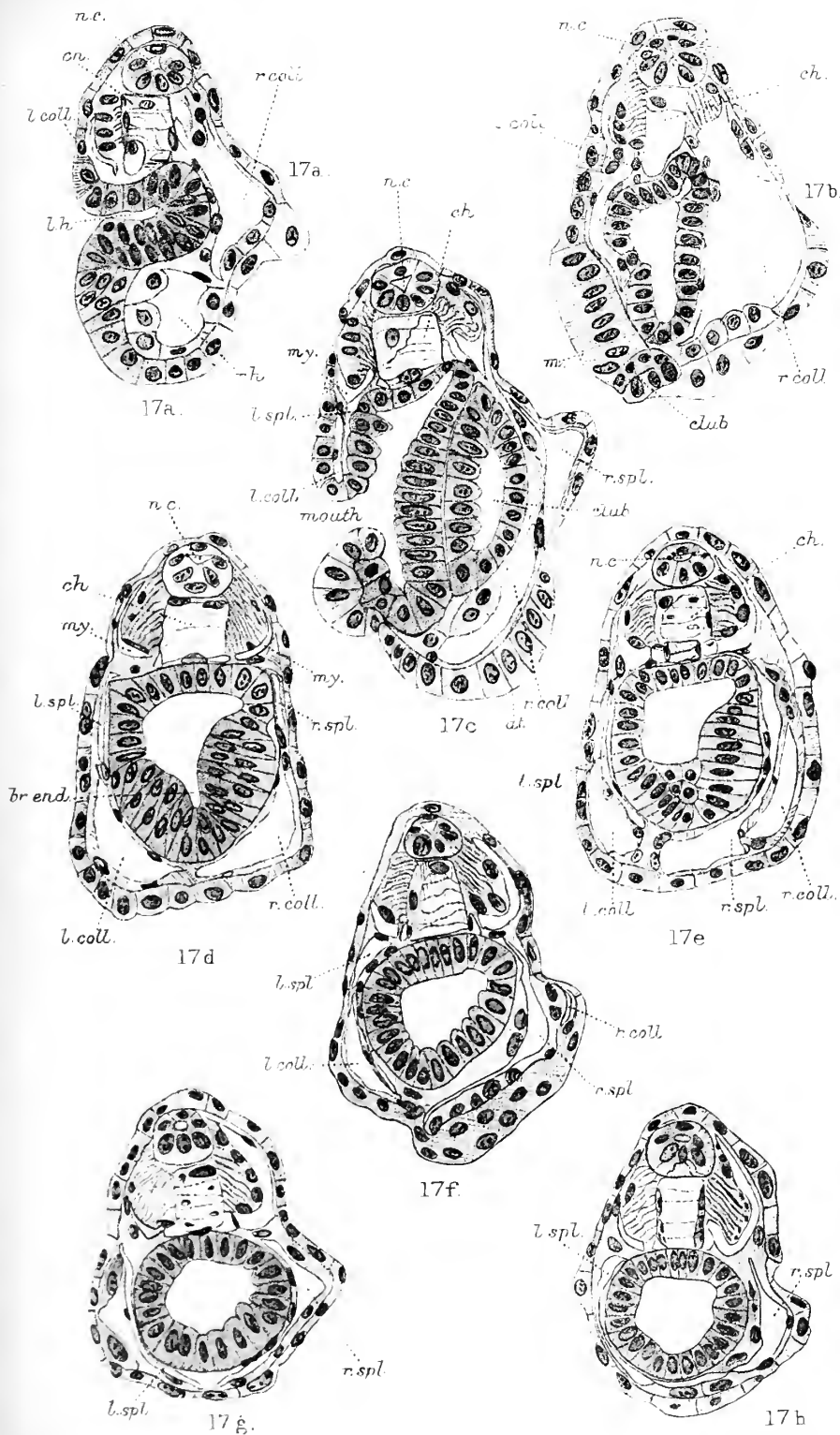


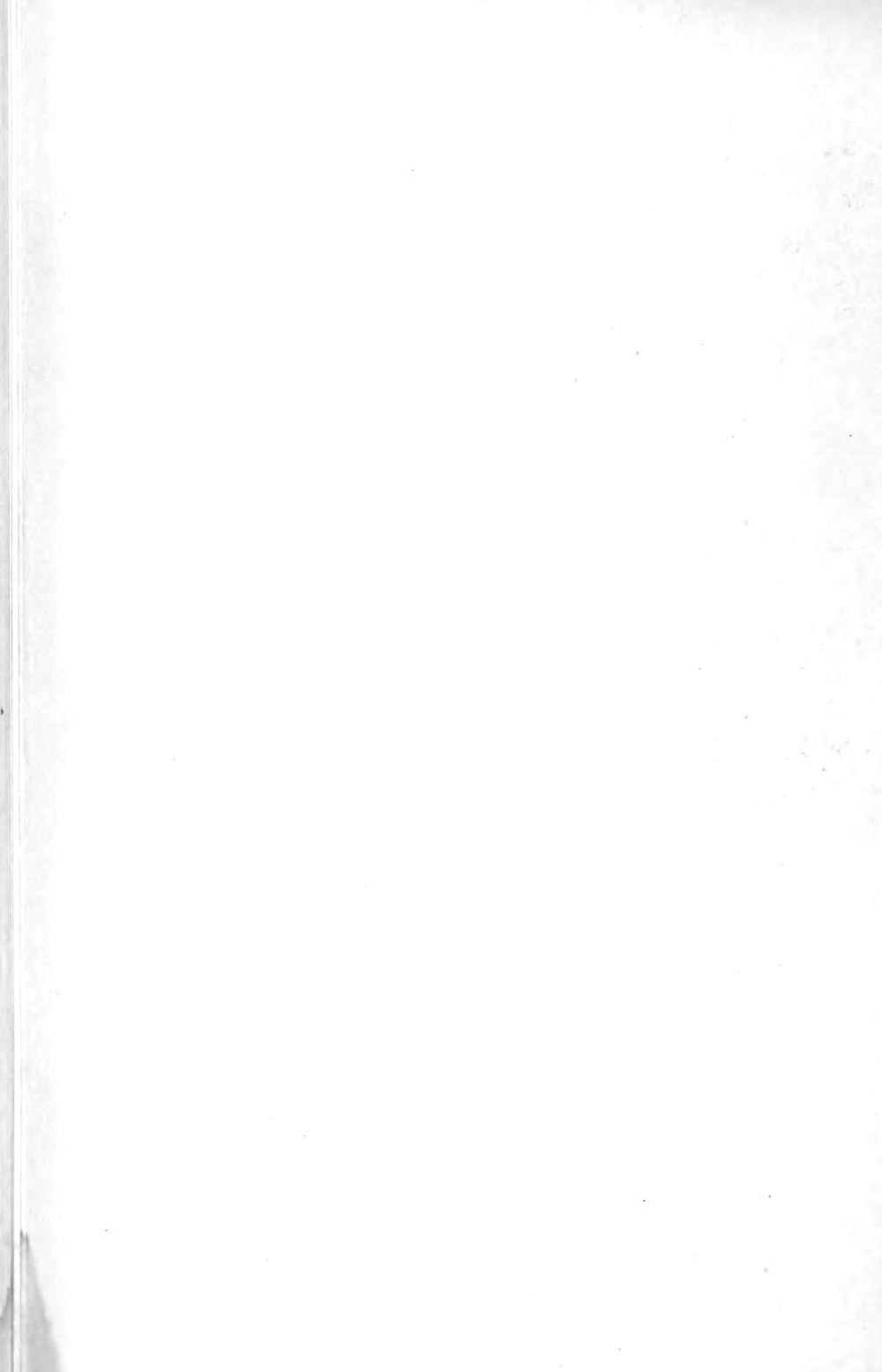
16f



16g







The Structure, Development, and Bionomics of the House-fly, *Musca domestica*, Linn.

Part III.—The Bionomics, Allies, Parasites, and the Relations
of *M. domestica* to Human Disease.

By

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Late Lecturer in Economic Zoology, University of Manchester.

With Plate 22.

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I. INTRODUCTION.

THE present paper concludes this study of the structure, development, and bionomics of *Musca domestica* (the previous parts were published in 1907 and 1908). In it I have described the bionomics, certain of its allies which may occur in houses, its parasites, and its relation to man, especially as the carrier of the bacilli of certain infectious diseases.

The last portion of the present paper, in which is described what is known concerning the ability of *M. domestica* and its allies to carry and disseminate the bacteria of many important diseases, shows, I hope, the grave character of its relation to man. Although its importance in this respect is being gradually realised in this country, it is not so widely recognised as it should be. In the United States of America it is proposed to change this insect's name from the house-fly to the "Typhoid fly"; notwithstanding certain objections to this name, it clearly indicates that more attention must be paid to preventive measures, that is, they must be reduced by the deprivation of suitable breeding-places. I have not discussed in the present paper the relation of house-flies to infantile or summer diarrhœa, chiefly because we are not yet certain as to the specific cause, but this disease may be included for the present under typhoid or enteric fever in so far as the relation of flies with it is concerned.

I should like to take this opportunity of thanking those medical men, whose names I mention later, for the kind manner in which they have replied to my inquiries concerning their observations on various diseases of which they have special knowledge.

II. DISTRIBUTION.

Musca domestica is probably the most widely distributed insect to be found; the animal most commonly associated with man, whom it appears to have followed over the entire globe. It extends from the sub-polar regions, where Linnæus refers to its occurrence in Lapland, and Finmark as “*rara avis in Lapponia*, at in Finmarchia Norwegiæ integras domos fere replet,” to the tropics, where it occurs in enormous numbers. Referring to its abundance in a house near Pará in equatorial Brazil, Austen (1904) says: “At the mid-day meal they swarmed on the table in almost inconceivable numbers,” and other travellers in different tropical countries have related similar experiences to me, how they swarm round each piece of food as it is carried to the mouth.

In the civilised and populated regions of the world it occurs commonly, and the British Museum (Natural History) collection and my own contain specimens from the following localities. Certain of the localities have, in addition, been obtained from lists of insect faunas:

Asia.—Aden; North West Provinces (India); Calcutta; Madras; Bombay (it probably occurs over the whole of India); Ceylon; Central China; Hong-Kong; Shanghai; Straits Settlements; Japan.

Africa.—Port Said; Suez; Egypt; Somaliland; Nyassaland; Uganda; British E. Africa; Rhodesia; Transvaal; Natal; Cape Colony; Madagascar; Northern and Southern Nigeria; St. Helena; Madeira.

America.—Distributed over North America; Brazil; Monte Video (Uruguay); Argentine; Valparaíso; West Indies.

Australia and New Zealand.

Europe and the isles of the Mediterranean; it is especially common in Cyprus.

Not only is this world-wide distribution of interest, but its distribution in our own country is noteworthy. From observations that I have made during a number of years in town and

suburban houses and country houses and cottages, I find that in the former it is by far the commonest house-fly. But whereas *M. domestica* may be almost the only species in warm places where food is present, such as restaurants and kitchens, in other rooms of houses *Homalomyia canicularis*, the small house-fly, increases in proportion and often predominates; occasionally one may find it to be commoner than *M. domestica*. In country houses the proportions vary by the intrusion of *Stomoxys calcitrans*, which I have often found to be the dominant species. In a certain country cottage, out of the several hundreds captured, *S. calcitrans* formed 50 per cent. of the total, the rest being chiefly *H. canicularis* together with *Anthomyia radicum*, whose larvæ, as I have shown (1907), breed in horse-manure with those of *M. domestica*. The following records taken from a "fly census" that was made in 1907 may be taken as illustrative of the proportional abundance of the different species in different situations; although the numbers of these records are small the proportions are more obvious.

Place.	<i>M. domestica</i> .	<i>H. canicularis</i> .	Other species.
Restaurant, Manchester .	1869	14	2 (<i>M. stabulans</i> , <i>C. erythrocephala</i>).
Kitchen, detached suburban house (six records), Lancashire	581	265	14
Kitchen, detached suburban house in Manchester	682	7	14
Stable, suburban house .	22	153	14 (12. <i>S. calcitrans</i>).
Bedroom, suburban house .	1	33	4 (<i>M. stabulans</i>).

Out of a total of 3856 flies caught in different situations, such as restaurants, kitchens, stables, bedrooms and hotels, 87·5 per cent. were *M. domestica*, 11·5 per cent. *H. canicularis*, and the rest were other species such as *S. calcitrans*, *Muscina stabulans*, *C. erythrocephala*, and *Anthomyia radicum*. These figures are comparatively small, but

are representative of the average occurrence, as I have observed, of the different species.

For the proportional occurrence in similar localities we have interesting figures given by Howard (1900) for the United States. Of 23,087 flies caught in rooms where food supplies are exposed he found that 22,808, or 98·8 per cent. of the whole number, were *M. domestica*, and of the remaining 1·2 per cent. *H. canicularis* was the commonest species. Hamer (1908) found that more than nine tenths of the flies caught in the kitchens and "living-rooms" of houses in the neighbourhood of depôts for horse-refuse, manure, etc., were *M. domestica*. In a further report Hamer gives more details as to the different species that were found. In one lot of 35,000 flies caught on four fly-papers exposed in similar positions, 17 per cent. were *Homalomyia canicularis*, less than 1 per cent. were *C. erythrocephala*, and considerably less than 1 per cent. were *Muscina stabulans*, whereas of nearly 6000 flies caught in another situation in four fly-balloons 24 per cent. were *H. canicularis*, 15 per cent. were *C. erythrocephala*, and nearly 2 per cent. were *M. stabulans*. He gives an interesting diagram showing from counts of flies the seasonal prevalence which I have previously recorded from observation. The report shows how the proportions of the different species vary in different situations according to the substances and refuse that are present in the locality. We may therefore say with certainty that *M. domestica* is the commonest species of house-fly, and next to this *H. canicularis*, and that in country houses *S. calcitrans* often occurs in large numbers, although it is not a house-fly in the strict sense of the word.

III. FLIES OCCURRING AS CO-INHABITANTS OF HOUSES WITH *M. DOMESTICA* OR AS VISITANTS.

We have seen from the preceding section that *M. domestica* is by far the commonest species which occurs in houses, and is, in fact, "domesticated" in the true sense of the word

—Linnaeus never selected a more truly specific title; nevertheless, other species of closely allied flies are found in houses. These may be either co-inhabitants, that is, living in houses, as in the case of *H. canicularis* and one or two others to be mentioned subsequently, or they may be visitants. The visitants normally lead an open-air life, but sometimes, as in the case of *Stomoxys calcitrans*, they spend a portion of their time in houses, when climatic conditions are less favourable for out-door life. Such flies as the blow-fly, or "blue-bottle," *Calliphora erythrocephala*, and its allies, enter houses only in search of suitable substances upon which to deposit their eggs. The appearance in houses of certain flies, as, for example, *Pollenia rudis*, can only be regarded as accidental, and the cause may be often traced to the occurrence of climbing plants such as ivy or other creepers on the walls of the house.

In India two species of flies closely allied to *M. domestica* are found—*Musca domestica* sub-sp. *determinata* Walker and *M. enteniata*, both of which, on account of their close resemblance to *M. domestica* and the similarity of their breeding habits, are frequently mistaken for it.

(1) *M. domestica* sub-sp. *determinata* Walker.

This Indian variety of the house-fly was described by Walker (1856) from the East Indies. His description is as follows: "Black, with a hoary covering; head with a white covering; frontalia broad, black, narrower towards the feelers; eyes bare; palpi and feelers black; chest with four black stripes; abdomen cinereous, with a large tawny spot on each side at the base; legs black; wings slightly grey, with a tawny tinge at the base; præbrachial vein forming a very obtuse angle at its flexure, very slightly bent inward from thence to the tip; lower cross-vein almost straight; alulæ whitish, with pale yellow borders; halteres tawny."

In appearance and size it is very similar to *M. domestica*. Its breeding habits are also similar. Aldridge (1904) states

that at certain seasons of the year it is present in enormous numbers. The method of disposal of the night soil is to bury it in trenches about one foot or less in depth. From one sixth of a cubic foot of soil taken from a trench at Meerut and placed in a cage, 4042 flies were hatched. Lieut. Dwyer collected 500 from one cage covering three square feet of a trench at Mhow. Specimens in the British Museum collection were obtained from the hospital kitchens, and Smith found them in a ward at Benares.

They have also been recorded from the N.W. Provinces, Kangra Valley (4500 feet), Dersa, and I have received specimens from Aden.

(2) *Musca enteniata* Bigot.

This fly has a distribution somewhat similar to the last species, and like it, has a marked resemblance to *M. domestica*, as Bigot's (1887) description indicates :

"Front très étroit, les yeux, toutefois, séparés. Antennis et palpes noirs ; face et joues blanches ; thorax noir avec trois larges bandes longitudinales grises ; flancs grisâtres, écusson noir avec deux bandes semblables ; cuillerons et balanciers d'un jaunâtre très pâle ; abdomen fauve, avec une bande dorsale noir et quelques reflets blancs ; pieds noirs ; ailes hyalines ; cinquième nervure longitudinal (Rondin) coudée suivant un angle légèrement arrondi, ensuite un peu concave ; deuxième transversale (l'extrême) presque perpendiculaire, légèrement bisinueuse, soudée à la cinquième longitudinale, à égale distance du conde et de la première nervure transversale (l'interne)."

M. enteniata measures 4 to 5 mm. in length. The British Museum collection contains specimens sent by Major F. Smith from Benares, with these notes : "Bred from human ordure ; hospital ward fly ; at an enteric stool ; bred from cow-dung fuel cakes." I have received specimens from Suez and Aden, and it is recorded as breeding in human excrement in Khartoum (Balfour, 1908) and in stable refuse, as also *M*

domestica and *M. corvina*. It will be seen, therefore, that its breeding habits are very similar to those of *M. domestica* and the sub-species *determinata*. It is interesting and important to note the rather exceptional choice of cow-dung as a breeding-place.

(3) *Homalomyia canicularis* L.

This species of fly (see 'Quart. Journ. Micr. Sci.,' vol. 51, Pl. 22, fig. 3) is often mistaken by the uninitiated for *M. domestica* which are not full grown. Although it may be called the small or lesser house-fly its differences from *M. domestica* are great, as it belongs to a different group of calypterate Muscidae, namely, the Anthomyidae. One of the chief distinguishing features of this group is that the fourth longitudinal vein of the wing (*M.* 1 + 2) goes straight to the margin of the wing and does not bend upwards at an angle as in *M. domestica*.

The male of *H. canicularis* differs from the female in some respects. In the male the eyes are close together, and the frontal region is consequently very narrow; the sides of this, these are the inner orbital regions, are silvery white, separated by a narrow black frontal stripe. In the female the space between the inner margins of the eyes is about one third of the width of the head; the frons is brownish black, and the inner orbital regions are dark ashy grey. The bristle of the antenna of *H. canicularis* is bare; in *M. domestica*, it will be remembered, the bristle bears a row of setae on its upper and lower sides. The dorsal side of the thorax of the male is blackish grey with three rather indistinct longitudinal black lines. In the female it is of a lighter grey, and the three longitudinal stripes are consequently more distinct. The abdomen of the male *H. canicularis* is narrow and tapering compared with that of *M. domestica*. It is bronze black in colour, and each of the three abdominal segments has a lateral translucent area, so that when it is seen against the light, as on a window-pane, three, and sometimes four, pairs of yellow translucent areas can be seen by the trans-

mitted light. In the female the abdomen is short in proportion to its length, and is of a greenish or brownish-grey colour.

H. canicularis appears in houses before *M. domestica*, and can be found generally in May and June. In the latter month its numbers are swamped, as it were, by *M. domestica*, and it appears to seek the other rooms of a house than the kitchen, although I have found it frequently in considerable numbers in kitchens. The average length is 5.7 mm.

The larva of *H. canicularis* (Pl. 22, fig. 1) is very distinct from that of *M. domestica*, as will be seen from the figure. It is compressed dorso-ventrally, and has a double row of processes on each side. Owing to the rough and spinous nature of these processes dirt adheres to the larva and gives it a dirty-brown appearance. The full-grown larva measures 5-6 mm. in length. The breeding habits of *H. canicularis* are very similar to those of *M. domestica*. The larvæ feed on waste vegetable substances and also on various excremental products, but particularly, I have found, on human excrement, for which they show a great partiality. I have frequently found excrement in privy middens to be a moving mass of the larvæ of *H. canicularis*. The larval period is from three to four weeks, and the insect spends fourteen to twenty-one days in the pupal stage.

(4) *Homalomyia scalaris* F.

Newstead (1907) has found this species occurring as a house-fly. It is slightly larger than, though similar in many respects to, *H. canicularis*. The larva is very similar in appearance. Newstead found the larvæ in ash-pit refuse, and bred the flies from human fæces. The larvæ have been found frequently to be the cause of intestinal myiasis.

(5) *Anthomyia radicum* Meigen.

This member of the Anthomyiidae has been found in houses, especially those in or near the country. The female has been illustrated already (Part I, 'Quart. Jour. Micr. Sci.,' vol. 51, Pl. 22, fig. 2). The male is darker in colour, the dorsal side

of the thorax being blackish with three black longitudinal stripes; the frontal region is very narrow; the abdomen is grey with a dark median stripe. The average length of the body is 5 mm.

In the summer they are common and may be found in the neighbourhood of manure. The eggs are laid in this substance, especially in horse-manure. The larvæ have also been found feeding on the roots of various cultivated cruciferous plants, from which the insect has derived the name "root-maggot." The eggs hatch out from eighteen to thirty-six hours after deposition. The first larval stadium lasts twenty-four hours, the second forty-eight hours, and five days later the larva changes into a pupa, the whole larval life occupying about eight days. The pupal stage lasts ten days, so that in warm weather the development may be completed in nineteen to twenty days. The full-grown larvæ measure 8 mm. in length, and may be distinguished by the tubercles surrounding the caudal extremity. In this species there are six pairs of spinous tubercles surrounding the posterior end and a seventh pair is situated on the ventral surface posterior to the anus. The tubercles of the sixth pair, counting from the dorsal side, are smaller than the rest and are bifid. The arrangement of the tubercles can be seen in fig. 2. The anterior spiracular processes (fig. 3) are yellow in colour and have thirteen lobes.

(6) *Stomoxys calcitrans* Linn.

The species is common, especially in the country from July to October, and during these months it may be often found in houses, although Hamer's observations (1908) appear to indicate that the presence of cowsheds, in which they occur in large numbers, does not affect their numbers in houses. I have found *S. calcitrans* in large numbers in the windows of a country house in March and April, and it may be found frequently out of doors on a sunny day in May, and throughout the ensuing summer months. It is normally an outdoor insect, but appears to seek the shelter

of houses, especially during wet weather, from which habit it has no doubt derived the popular name of "storm-fly"; it is also known as the "stable-fly." As these names may be equally applicable to certain other Diptera they should be discarded.

As I have already mentioned this species is frequently mistaken by the public for *M. domestica*, which is supposed to have adopted the biting habit, although the latter is unable to inflict the slightest prick. If examined side by side the great differences between the two will be seen readily (see Part I in 'Quart. Journ. Micr. Sci., vol. 51, Pl. 22, fig. 4). *S. calcitrans* has an awl-like proboscis for piercing and blood-sucking; this projects horizontally forward from beneath the surface of the head (fig. 4). It is slightly larger and more robust than *M. domestica*; the bristles of the antennæ bear setæ on their upper sides only. The colour is brownish with a greenish tinge; the dorsal side of the thorax has four dark longitudinal stripes, the outermost pair being interrupted. At the anterior end of the dorsal side of the thorax the medium light-coloured stripe has a golden appearance, which is very distinct when the insect is seen against the light. The abdomen is broad in proportion to its length, and each of the large second and third segments has a single median and two lateral brown spots; there is also a median spot on the fourth segment.

The life-history of *S. calcitrans* has been studied by Newstead (1906), and I have been able to confirm his observations during 1907 and 1908. From fifty to seventy eggs, measuring 1 mm. in length, are laid by the female. The eggs are laid on warm, decaying vegetable refuse, especially in heaps of fermenting grass cut from lawns; I have frequently confirmed this observation of Newstead's. The eggs are also deposited on various excremental substances upon which the larvæ feed. Osborne (1896) reared them in horse-manure; Howard (1900) states that they live in fresh horse-manure, and records their occurrence in outdoor privies in some localities; Newstead reared them in moist sheep's dung; they can also be reared in cow-dung.

The larvæ are creamy-white in colour and have a shiny, translucent appearance. They are rather similar to those of *M. domestica*, but can be distinguished by the character of the posterior spiracles. These (fig. 5 and 6) are wider apart than in *M. domestica* and are triangular in shape with rounded corners; each of the corners subtends a space in which a sinuous aperture lies. The centre of the spiracle is occupied by a circular plate of chitin. The anterior spiracular processes are five-lobed. Under warm conditions Newstead found that the egg state lasted from two to three days; the larval stage lasts from fourteen to twenty-one days and the pupal stage nine to thirteen days. There are three larval stages. The whole life-history may be complete in twenty-five to thirty-seven days. Some specimens passed the winter in the pupal state.

Although *S. calcitrans* does not frequent to such a great extent as *M. domestica* material likely to contain pathogenic intestinal bacilli, on account of its blood-sucking habits, which cause it to attack cattle and not infrequently man, it may occasionally transfer the anthrax bacillus, as many have believed, and give rise to malignant pustule, etc.

(7) *Calliphora erythrocephala* Mg.

This is the commoner of the two English blow-flies or "blue-bottles." The other species, *Calliphora* (*Musca*) *vomitaria*, is less common, although the name is frequently given to both species indiscriminately. They can be distinguished, however, by the fact that in *C. erythrocephala* the genæ are fulvons to golden-yellow and are beset with black hairs, whereas in *C. vomitaria* the genæ are black and the hairs are golden-red.

The appearance of *C. erythrocephala* is sufficiently well known with its bluish-black thorax and dark metallic blue abdomen. Its length varies from 7 to 13 mm. The larvæ are necrophagous. The flies deposit their eggs on any fresh or decaying meat, nor is such flesh always dead. On one occasion, when obtaining fresh material in the form of wild

rabbits upon which to rear the larvæ of *C. erythrocephala*, I found the broken leg of a live rabbit, which had been caught in a spring trap set the previous evening, a living mass of small larvæ, which were devouring the animal while it was still alive. An enormous number of eggs are laid by a single insect; Portchinski ('Osten. Sacken,' 1887) found from 450 to 600 eggs, though I have not found so many. With an average mean temperature of 23° C. (73·5° F.) and using fresh rabbits as food for the larvæ, the following were the shortest times in which I reared *C. erythrocephala*. The eggs hatched from ten to twenty hours after deposition. The larvæ underwent the first ecdysis eighteen to twenty-four hours after hatching; the second moult took place twenty-four hours later, and the third larval stage lasted six days, the whole larva life being passed in seven and a half to eight days. Fourteen days were spent in the pupal state; thus the development was complete in twenty-two to twenty-three days. I have no doubt that this time could be shortened by the presence of a very plentiful supply of food, as an enormous amount, comparatively, is consumed.

The full-grown larva may measure as much as 18 mm. in length. The posterior extremity is surrounded by six pairs of tubercles arranged as shown in the figure (fig. 12); there is also a pair of anal tubercles. The anterior spiracular (fig. 11) processes are nine-lobed. The posterior spiracles (fig. 10) are circular in shape and contain three slit-like apertures. In the second larval instar (fig. 9) there are only two slits in each of the posterior spiracles, and in the first larval instar (fig. 8) each of the posterior spiracles consists of a pair of small slit-like orifices. Howard (1900) found the fly on fresh human fæces, and Riley records it as destroying the Rocky Mountain locust.

C. erythrocephala is an outdoor fly, but frequently enters houses in search of material upon which to deposit its eggs and also for shelter. From its habit of frequenting fæces, which may be observed in this country especially in insanitary court-yards, and such food as meat and fruit, it is not improb-

able that it occasionally may bear intestinal bacilli on its appendages or body and thus carry infection. Its flesh-seeking habits may also render it liable to carry the bacilli of anthrax should it have access to infected flesh.

(8) *Muscina (Cyrtoneura) stabulans* Fallen.

This common species is frequently found in and near houses. I have usually found it occurring with *H. canicularis* in the early summer (June) before *M. domestica* has appeared in any numbers. It is larger than *M. domestica*, and more robust in appearance. Its length varies from 7 to nearly 10 mm. Its general appearance is grey. The head is whitish-grey with a "shot" appearance. The frontal region of the male is velvety black and narrow; that of the female is blackish-brown, and is about a third of the width of the head. The bristle of the antenna bears setæ on the upper and lower sides. The dorsal side of the thorax is grey and has four longitudinal black lines; the scutellum is grey. The abdomen, as also the thorax, is really black covered with grey; in places it is tinged with brown, which gives the abdomen a blotched appearance. The legs are rather slender, and are reddish-gold or dirty orange and black in colour.

The eggs are laid upon the following substances, on which the larvæ feed: Decaying vegetable substances such as fungi, fruit, cucumbers, decaying vegetables, and they sometimes attack growing vegetables, having been introduced probably as larvæ with the manure, as they also feed on rotting dung and cow-dung. Howard (l. c.) found the fly frequenting human excrement, and observed the species breeding in the same. In the United States it has been reared from the pupæ of the cotton-worm and the gipsy moth; Riley was of the opinion that in the first case it fed on the rotten pupæ only. In 1891 it was also reared on the masses of larvæ and pupæ of the elm-leaf beetle. Other observers record it as being reared from the pupæ of such Hymenoptera as *Lophyrus*. In all these cases of its occurrence in the pupæ of insects, it

is difficult to say whether it is parasitic or whether it feeds on the rotting pupæ only ; many observers are inclined to take the last view. The larva may reach a length of 11 mm. It is creamy-white in colour ; the anterior spiracular processes are five-lobed and are like hands from which the fingers have been amputated at the first joint. The posterior spiracles are rounded and enclose three triangular-shaped areas, each containing a slit-like aperture. I have not been able to study the complete life-history, but Taschenberg (l. c.) states that it occupies five or six weeks.

(9) *Lucilia Cæsar* L.

Although it is not a house-fly, this common fly occasionally occurs in houses, especially those in the country, and it is often called a "blue-bottle." It is smaller than *C. erythrocephala* and is more brilliant in colouring, being of a burnished gold, sometimes bluish, and also of a shining green colour.

It frequents the excrement of man and other animals in which it is able to breed. Howard (l. c.) reared it from human excrement. It also breeds in carrion, but the chief breeding-place in which I have found it in this country is on the backs of sheep. It is one of the destructive species of "maggots" of sheep. The larvæ are very similar to those of *C. erythrocephala*—in fact, Portchinski considered them indistinguishable from the larvæ of the latter except in size. The full-grown larva measures 10–11 mm. in length. The larval life lasts about fourteen days, and the pupal stage a similar length of time, but I have reason to believe that under very favourable conditions development may take place in a much shorter time.

(10) *Psychoda* spp.

There may be found frequently on window-panes small, grey, moth-like flies belonging to the family Psychodidæ. The wings of these small flies are large and broad in proportion to the size of the body, and are densely covered with hair ; when the insect is at rest they slope in a roof-like

manner. The larvæ of some species breed in human and other excrement, others breed in decaying vegetable substances, while certain species breed in water, especially when polluted with sewage, and these aquatic species have the spiracular apparatus modified accordingly. Although a form, *Phlebotomus*, which occurs in Southern Europe, has blood-sucking habits, the British species have no such annoying habits, and are of little importance in their relation to man.

IV. PHYSIOLOGY.

1. The Influence of Food, Temperature, and Light.

Food.—Mention has already been made in the second part of this work of the influence of food on the development of the larvæ; the experiments which were carried out showed that the larvæ develop more rapidly in certain kinds of food, such as horse-manure, than in others. It has yet to be discovered what are the chemical constituents which favour the more rapid development. It was found that insufficient food in the larval state retarded development and produced flies which were subnormal in size. Bogdanow (1908), in an interesting experiment, fed *M. domestica* through ten generations on unaccustomed food such as meat and tanacetum in different proportions, and he found that the resulting flies did not show any change.

Temperature.—The influence of temperature on the development of the larvæ has been shown also. A high temperature accelerates the development of the egg, larva and pupa. Temperature also affects the adult insect; they are most active at a high summer temperature, and cold produces an inactive and torpid condition. They are able, however, to withstand a comparatively low temperature. Bachmetjew (1906) was able to submit *M. domestica* to as low a temperature as -10°C. , and vitality was retained, as they recovered when brought into ordinary room temperature. Donhoff (1872) performed a number of experiments previous

to this with interesting results. He submitted *M. domestica* for five hours to a temperature of -1.5°C ., and they continued to move. Exposed for eight hours to a temperature of first -3°C . and then -2°C . they moved their legs. On being submitted for twelve hours to a temperature first of -3.7°C . and then -6.3°C ., they appeared to be dead, but on being warmed they recovered. When exposed for three hours to a temperature of -10°C . which was then raised to -6°C ., they died. These experiments show that *M. domestica* is able to withstand a comparatively low degree of temperature.

Light.—The female of *M. domestica* deposits the eggs in dark crevices of the substance chosen for the larval nidus and as far away from the light as possible. Bécларd (1858) showed that the eggs develop more quickly under blue and violet glass than under red, yellow, green, or white. The larvæ are negatively heliotropic, as Loeb (1890) has also proved in the larvæ of the blow-fly. As I have previously shown, the distinction between light and darkness is probably appreciated by the larvæ by means of the sensory tubercles of the oral lobes.

2. Hibernation.

This question is intimately connected with the preceding physiological facts. The disappearance of the flies towards the end of October and in November is a well-known fact, and an endeavour to discover the reason for this has been made in the present investigation.

I have found that the majority of flies observed were killed off by the fungus *Empusa muscæ* Cohn which is described in the present paper. Of the remainder some hibernate and some die naturally. This natural death may be compared, I think, to the like phenomenon that occurs in the case of the hive-bee *Apis mellifica*, where many of the workers die at the end of the season by reason of the fact that they are simply worn out, their function having been fulfilled. The flies which die naturally have probably lived for many

weeks or months during the summer and autumn, and in the case of the females have deposited many batches of eggs; their life work, therefore, is complete. Those flies which hibernate are, I believe, the most recently emerged, and therefore the youngest and most vigorous. On dissection it is found that the abdomens of these hibernating individuals are packed with fat cells, the fat body having developed enormously. The alimentary canal shrinks correspondingly and occupies a very small space; this is rendered possible by the fact that the fly does not take food during this period. In some females it was found that the ovaries were very well developed, while in others they were small, and mature spermatozoa were found in the males. Like most animals in hibernating, *M. domestica* becomes negatively heliotropic and creeps away into a dark place. In houses they have been found in various kinds of crevices such as occur between the woodwork and the walls. A favourite place for hibernation is between wall-paper which is slightly loose and the wall. A certain number hibernate in stables, where, owing to the warmth, they do not become so inactive, and they emerge earlier at the latter end of spring. During the winter the hibernating flies are sustained by means of the contents of the fat body, which is found to be extremely small in hibernating flies if dissected when they first emerge in May and June. The abdominal cavity is at first considerably decreased in size, but the fly begins to feed and soon the alimentary tract regains its normal size, and, together with the development of the reproductive organs, causes the abdomen to regain its normal appearance. The emergence from hibernation appears to be controlled by temperature, as one may frequently find odd flies emerging from their winter quarters on exceptionally warm days in the early months of the year (see Appendix).

3. Flight.

The distance that *M. domestica* is able to fly is one of practical importance in connection with their breeding habits

and disease-germ-carrying powers. Normally they do not fly great distances. They may be compared to domestic pigeons which hover about a house and the immediate neighbourhood. On sunny days they may be found in large numbers out-of-doors, but they retire into the houses when it becomes dull or rains. They are able to fly, however, a considerable distance, and can be carried by the wind. A few years ago, when visiting the Channel Islands, I found *M. domestica* from $1\frac{1}{2}$ to 2 miles from any house or any likely breeding-place, so far as I was able to discover. Dr. M. B. Arnold has made some exact experiments at the Monsall Fever Hospital, Manchester, on the distance travelled by flies.¹ Three hundred flies were captured alive, and marked with a spot of white enamel on the back of the thorax. These were liberated in fine weather. Out of the 300 five were recovered in fly-traps at distances varying from 30 to 190 yards from the place of liberation, and all the recoveries were within five days. *M. domestica* is also able to fly at a considerable height above ground, and I have found them flying at an altitude of 80 feet above the ground. Such a height would greatly facilitate their carriage by the wind.

4. Regeneration of Lost Parts.

If the wings or legs of *M. domestica* are broken off they do not appear to be able to regenerate the missing portions, as in the case of some insects, notably certain Orthoptera. Kammerer (1908), however, experimenting with *M. domestica* and *C. vomitoria*, has found that if the wing is extirpated from a recently pupated fly it is occasionally regenerated. The new wing is at first homogeneous, and contains no veins, but these appear subsequently.

¹ Recorded on p. 262 of the 'Report on the Health of the City of Manchester for 1906,' by James Niven, 1907.

V. NATURAL ENEMIES AND OCCASIONAL PARASITES.

The most important of all the natural enemies of *M. domestica* is the parasitic fungus *Empusa muscæ*, which will be described here; this is the most potent of the natural means of destruction. Of animals, apart from the higher animals such as birds, spiders probably account for the greatest number, though owing to the normally clean condition of the modern house these enemies of the house-fly are refused admittance. I have been unable to rear any insect parasites, such as ichneumonids, from *M. domestica*. Their life indoors and the cryptic habits of the larvæ no doubt save them from the attacks of such insects; but Packard (1874) records the occurrence of the pupa of what was probably a Dermestid beetle, which he figures; this was found in a pupa of *M. domestica*. Predatory beetles and their larvæ probably destroy the larvæ, and Berg (1898) states that a species of beetle, *Trox suberosus* F., known as "Champi" in S. America, is an indirect destructor of the common fly. I have frequently observed the common wasp, *Vespa germanica*, seize *M. domestica* and carry it away. In some places in India it is the custom, so I have been told by residents, to employ a species of Mantis, one of the predatory "praying insects," to destroy the house-flies.

In view of the fact that the Arachnids *Chernes nodosus* and the species of Gamasid are occasionally found actually attached in a firm manner to *M. domestica*, they will be described under this head, but it must be clearly understood that it is still an open question whether they are external parasites in the true sense of the word, or whether *M. domestica*, instead of being the host, is merely the transporting agent as it appears to be in the majority of cases. For the present they may be termed for convenience "occasional parasites," in view of the fact that they have been found occasionally feeding upon *M. domestica*.

1. *Chernes nodosus* Schrank.

There are frequently found attached to the legs of the house-fly small scorpion or lobster-like creatures which are Arachnids belonging to the order Pseudo-scorpionidea; the term "chelifers" is also applied to them on account of the large pair of chelate appendages which they bear. The species which is usually found attached to *M. domestica* is *Chernes nodosus* Schrank (fig. 13). It is very widely distributed, and my observations agree with those of Pickard-Cambridge (1892), who has described the group.

The species is 2.5 mm. in length and Pickard-Cambridge's description of it is as follows:

"Cephalothorax and palpi yellowish red-brown, the former rather duller than the latter. Abdominal segments yellow-brown; legs paler. The caput and first segment of the thorax are of equal width (from back to front); the second segment of the thorax is very narrow. The surface of the cephalothorax and abdominal segments is very finely shagreened, the latter granulose on the sides. The hairs on this part as well as on the palpi and abdomen are simple, but obtuse. The palpi are rather short and strong. The axillary joint is considerably and somewhat subconically protuberant above as well as protuberant near its base underneath. The humeral joint at its widest part, behind, is considerably less broad than long; the cubital joint is very tumid on its inner side; the bulb of the pincers is distinctly longer, to the base of the fixed claw, than its width behind; and the claws are slightly curved and equal to the bulb in length."

They appear to be commoner in some years than in others. Godfrey (1909) says: "The ordinary habitat of *Ch. nodosus*, as Mr. Wallis Kew has pointed out to me, appears to be among refuse, that is, accumulations of decaying vegetation, manure-heaps, frames and hot-beds in gardens. He refers to its occurrence in a manure-heap in the open air at Lille, and draws my attention to its abundance in a melon-frame near Hastings in 1898, where it was found by Mr. W. R. Butterfield." In

view of these facts it is not difficult to understand its frequent occurrence on the legs of flies, which may have been on the rubbish heaps either for the purpose of laying eggs, or, what is more likely, because they have recently emerged from pupæ in those places and in crawling about, during the process of drying their wings, etc., their legs were seized by the *C. nodosus*.

The inter-relation of the *Chernes* and *M. domestica*, however, is one of no little complexity; much has been written and many diverse views are held concerning it. An interesting historical account of the occurrences of these Arachnids on various insects has been given by Kew (1901). Three views are held in explanation of the association and they are briefly these: First, that the *Chernes*, by clinging passively to the fly, uses it as a means of transmission and distribution; second, that the Arachnid is predaceous; and third, that it is parasitic on the fly. Owing to the unfortunate absence of convincing experimental proof in favour of either of the last two opinions, it is practically impossible to give any definite opinion as to the validity of these views; nevertheless they are worthy of examination.

The dispersal theory was held by Pickard-Cambridge and Moniez (1894). Whether the other views are held or not there is no doubt that such an association, even if it were only accidental, would result in a wider distribution of the species of *Chernes*, as the flies are constantly visiting fresh places suitable as a habitat for the same. Except in one or two recorded cases the Arachnids are always attached to the legs of the fly, the chitin of which is hard and could not be pierced, a fact which is held in support of this theory as the only explanation of the association.

The parasitic and predaceous views are closely related. The *Pseudo-scorpionidea* feed upon small insects, which they seize with their chelæ. It is suggested by some that the *Chernes* seizes the legs of the fly without realising the size of the latter. Notwithstanding its size, however, they remain attached until the fly dies and then feed upon the

body. In some cases as many as ten of the Arachnids have been found on a single fly, and if the movements of the insect are impeded by the presence of a number of the Chernes it will be easily understood that the life of the fly will be curtailed thereby. Pseudo-scorpionidea have been observed feeding on the mites that infest certain species of Coleoptera, and it has been suggested that they associated with the flies for the same purpose, although I do not know of any recorded case of a fly infested with mites carrying Chernes also. If this were the case the Chernes would be a friend and not a foe of the fly, as Hickson (1905) has pointed out.

There are few records to support the view that the Chernes is parasitic on the fly. Donovan (1797) mentions the occurrence of a pseudo-scorpionid on the body of a blow-fly, and Kirby and Spence (1826) refer to their being occasionally parasitic on flies, especially the blow-fly, under the wings of which they fix themselves. It is probable that the Chernes seldom reaches such a position of comparative security on the thorax of the fly; should it succeed in doing so, however, it could become parasitic in the true sense of the word. As I have previously pointed out, little experimental evidence is at present available and further investigation is necessary before it is possible to maintain more than a tentative opinion with regard to this association between the Chernes and the fly. It is obvious that the association will result in the distribution of the Pseudo-scorpionid, but whether this is merely incidental and the real meaning lies in a parasitic or predaceous intention on the part of the Arachnid, as some of the observations appear to indicate, further experiments alone will show.

2. Acarina or Mites borne by House-flies.

As early as 1735 de Geer observed small reddish Acari in large numbers on the head and neck of *M. domestica*. They ran about actively when touched. The body of this mite was oval in shape, completely chitinised, and polished;

the dorsal side was convex and the ventral side flat. Linnæus (1758) called this mite *Acarus muscarum* from de Geer's description, and Geoffroy (1764) found what appears to be the same, or an allied species of, mite, which he called the "brown fly-mite." Murray (1877) describes a form, *Trombidium parasiticum*,¹ which is a minute blood-red mite parasitic on the house-fly. He says: "In this country they do not seem so prevalent, but Mr. Riley mentions that in North America, in some seasons, scarcely a fly can be caught that is not infested with a number of them clinging tenaciously round the base of the wings." As it only possessed six legs it was doubtless a larval form.

Anyone who has collected Diptera as they have emerged from such breeding-places as hot-beds, rubbish and manure heaps will have noticed the frequently large number of these insects which are to be found carrying immature forms of the Acari. These are being transported merely by the flies in the majority of cases. Mr. Michael tells me that he used to call such flies "the emigrant waggons"—a very descriptive term. Many of these mites belong to the group Gamasidæ—the super-family Gamasoidea of Banks (1905). These mites have usually a hard coriaceous integument. In shape they are flat and broad and have rather stout legs. Sometimes immature forms of these mites swarm on flies emerging from rubbish heaps. Banks holds the opinion that they are not parasitic, but that the insect is only used as a means of transportation. It is difficult to decide whether this is so in all cases. I have illustrated (fig. 14) a specimen of the small house-fly, *H. canicularis*, caught in a room; on the under-side of the fly's abdomen a number of immature Gamasids² are attached,

¹ This species was named *Atoma parasiticum* and later *Astoma parasiticum* by Latreille ('Magazin Encyclopedique,' vol. iv. p. 15. 1795). Mr. A. D. Michael tells me that the genus was founded on *Trombidium parasiticum* of de Geer. They were really larval Trombididæ and *Atoma* was founded on larval characters; probably any larval *Trombidium* came under the specific name.

² Being unable to identify these immature specimens I submitted them to Mr. Michael, who kindly informs me that it is extremely diffi-

apparently by their stomal regions. These specimens may be truly parasitic, as I am inclined to believe, since many Acari are parasitic in the immature state, although the adults may not be so; on the other hand this form of attachment may be employed as a means of maintaining a more secure hold of the transporting insect.

3. Fungal Disease—*Empusa muscæ* Cohn.

Towards the end of the summer large numbers of flies may be found attached in a rigid condition to the ceiling, walls or window-panes. They have an extremely life-like appearance, and it is not until one examines them closely or has touched them that their inanimate, so far as the life of the fly is concerned, condition is discovered. These flies have been killed by the fungus *Empusa muscæ* Cohn, and in the later stages of the disease its fungal nature is recognised by the fact that a white ring of fungal spores may be seen around the fly on the substratum to which it is attached. The abdomen of the fly is swollen considerably, and white masses of sporogenous fungal hyphæ may be seen projecting for a short distance from the body of the fly, between the segments, giving the abdomen a transversely striped black and white appearance.

The majority of flies which die in the late autumn—and it is then that most of the flies which have been present during the summer months perish—are killed by this fungus. Its occurrence, therefore, is of no little economic value, especially if it were possible to artificially cultivate it and destroy the flies in the early summer instead of being compelled to wait until the autumn for the natural course of events.

Empusa muscæ belongs to the group Entomophthoreæ, the members of which confine their attacks to insects, and in many cases, as in the case of the present species, are productive of great mortality among the individuals of the species of

cult to identify immature Gamasids owing to the scarcity of knowledge as to their life-histories, but he says that they are very like *Dinychella asperata* Berl.

insect attacked. In this country it may be found from about the beginning of July to the end of October, and usually occurs indoors. It appears to be very uncommon out-of-doors. A case has been recently recorded¹ of its occurrence on Esher Common, where it had attacked a species of Syrphid, *Melanostomum scalare* Fabr. Thaxter (1888) also mentions two cases of its occurrence out-of-doors in America, in both of which cases it had attacked, singularly enough, species of Syrphidæ. This author states that *Empusa muscæ* is probably the only species which occurs in flowers attractive to insects, but he only observed it on the flowers of *Solidago* and certain Umbellifereæ.

The development of this species was studied by Brefeld (1871). An *Empusa* spore which has fallen on a fly rests among the hairs covering the insect's body and there adheres. A small germinating hypha develops, which pierces the chitin, and after entering the body of the victim penetrates the fat-body. In this situation, which remains the chief centre of development, it gives rise to small spherical structures which germinate in the same manner as yeast cells, forming gemmæ. These separate as they are formed, and falling into the blood sinus are carried throughout the whole of the body of the fly. It was probably these bodies that Cohn (1855) found, and he explained their presence as being due to spontaneous generation; he believed that the fly first became diseased and that the fungus followed in consequence. After a period of two or three days the fly's body will be found to be completely penetrated by the fungus, which destroys all the internal tissues and organs. The whole body is filled with the gemmæ, which germinate and produce ramifying hyphæ (fig. 15). The latter pierce the softer portions of the body-wall between the segments and produce the short, stout conidiophores (*c.*), which are closely packed together in a palisade-like mass to form a compact white cushion of conidiophores, which is the transverse white ring that one finds between each of the segments of a diseased, and

¹ 'Trans. Ent. Soc. London,' 1908 ("Proceedings," p. 57).

consequently deceased, fly. A conidium now develops (fig. 16) by the constriction of the apical region of the conidiophore. When it is ripe the conidium (fig. 17) is usually bell-shaped, measuring $25-30\ \mu$ in length; it generally contains a single oil-globule (*o.g.*). In a remarkable manner it is now shot off from the conidiophore, often for a distance of about a centimetre, and in this way the ring or halo of white spores, which are seen around the dead fly, are formed. In some cases, although I find that it is not an invariable rule as some would suggest, the fly, when dead, is attached by its extended proboscis to the substratum. Giard (1879) found that blow-flies killed by *Entomophthora calliphora* were attached by the posterior end of the body. If the conidia, having been shot off, do not encounter another fly, they have the power of producing a small conidiophore, upon which another conidium is in turn developed and discharged. If this is unsuccessful in reaching a fly a third conidium may be produced, and so on. By this peculiar arrangement the conidia may eventually travel some distance, and it is no doubt a great factor in the wide distribution of the fungus, once it occurs. On the fly itself short conidiophores may be found producing secondary conidia.

Reproduction by conidia appears to be the only form of generation, as we are still uncertain as to the occurrence of a resting-spore stage in this species. Winter (1881) states that he found resting-spores in specimens of *M. domestica* occurring indoors; they also produced conidia which he identified as *E. muscæ*. These azygospores measured $30-50\ \mu$ in diameter, and were produced laterally or terminally from hyphæ within the infected fly. Giard (*l. c.*) describes resting spores which were produced externally and on specimens found in cool situations. Brefeld, however, is of the opinion that *E. muscæ* does not produce resting-spores. The question of the production of resting-spores needs further investigation, as it is one of some importance. In the absence of confirmatory evidence it is extremely difficult to understand how the gap in the history of the *Empusa*, between the

late autumn of one year and the summer of the next, is filled. A number of suggestions have been made, many of which cannot be accepted; for example, Brefeld believes that the *Empusa* is continued over the winter in warmer regions, migrating northwards with the flies on the return of summer! In the case of *Entomophthora calliphora*, Giard believes that the cycle is completed by the corpses of the blow-flies falling to the ground, when the spores might germinate in the spring and give rise to conidia which infect the larvæ. Olive (1906) studied the species of *Empusa* which attacks a species of *Sciara* (Diptera) and found the larvæ infected. He accordingly thinks that the disease may be carried over the winter by those individuals which breed during that period in stables and other favourable places. As I have shown, *M. domestica*, under such favourable conditions as warmth and supply of suitable larval food, is able to breed during the winter months, although it is not a normal occurrence so far as I have been able to discover. If, then, these winter-produced larvæ could become infected they might assist in carrying over the fungus from one year to the next, and thus carry on the infection to the early summer broods of flies. This suggestion and the possible occurrence of a resting-spore stage appears to me to be the probable means by which the disease may be carried over from one "fly-season" to the next.

E. muscæ, besides occurring in *M. domestica*, has been found on several species of *Syrphidæ*, upon which it usually occurs out-of-doors, as I have already mentioned. In addition to these Thaxter records its occurrence in *Lucilia cæsar* and *Calliphora vomitoria*.

VI. TRUE PARASITES.

1. Flagellata. *Herpetomonas muscæ-domesticæ* Burnett.

This flagellate has been known as a parasite of the alimentary tract of *M. domestica* for many years. Stein (1878) figures a flagellate which he calls *Cercomonas muscæ-domestica*, and identifies it with the *Bodo muscæ-*

domesticæ described by Burnett and the *Cercomonas muscarum* of Leidy. For this form figured by Stein, a new genus, *Herpetomonas*, was instituted by Kent (1880-81), and it is taken as the type-species. It was not until the economic importance of certain of the hæmo-flagellates was recognised that other flagellates, including *H. muscæ-domesticæ*, received further attention, and then Prowazek (1904) described with great detail the development of this species. In the previous year Léger (1903) had given a short account of it, and since Prowazek's memoir Patton (1908, 1909) has given short preliminary accounts of his study of the life-history. The accounts of both these authors differ in several respects from that of Prowazek, as will be shown. I have examined a very large number of the contents of English specimens of *M. domestica*, but, with one or two doubtful exceptions, unfortunately I have been unable so far to discover any of these flagellates in my film preparations.

The full-grown flagellate (VIII) measures 30-50 μ in length. The body is flattened and lancet-shaped, the posterior end being pointed and the anterior end bluntly rounded. The alveolar endoplasm contains two nuclear structures. In the centre is the large "trophonucleus" (*tr.*); it contains granules of chromatin, but is sometimes difficult to see. Near the anterior end the deeply staining rod-shaped "kinetonnucleus" (blepharoplast of many authors) (*k.*) lies, usually in a transverse position. The single stout flagellum, which is a little longer than the body of the flagellate, arises from the anterior end, near the kinetonnucleus. Prowazek describes the flagellum as being of a double nature and having a double origin; this, which is a mistaken interpretation, is repeated by Lingard and Jennings (1906).

This mistake, as pointed out by Léger and Patton, is due to the fact that the majority of the adult flagellates have the appearance of a double flagellum, which represents the beginning of the longitudinal division of the flagellate (VI). Patton (1908) figures a stage in *H. lygæi* with the double flagellum, and Léger (1902) in a similar stage in *H. jaculum*,

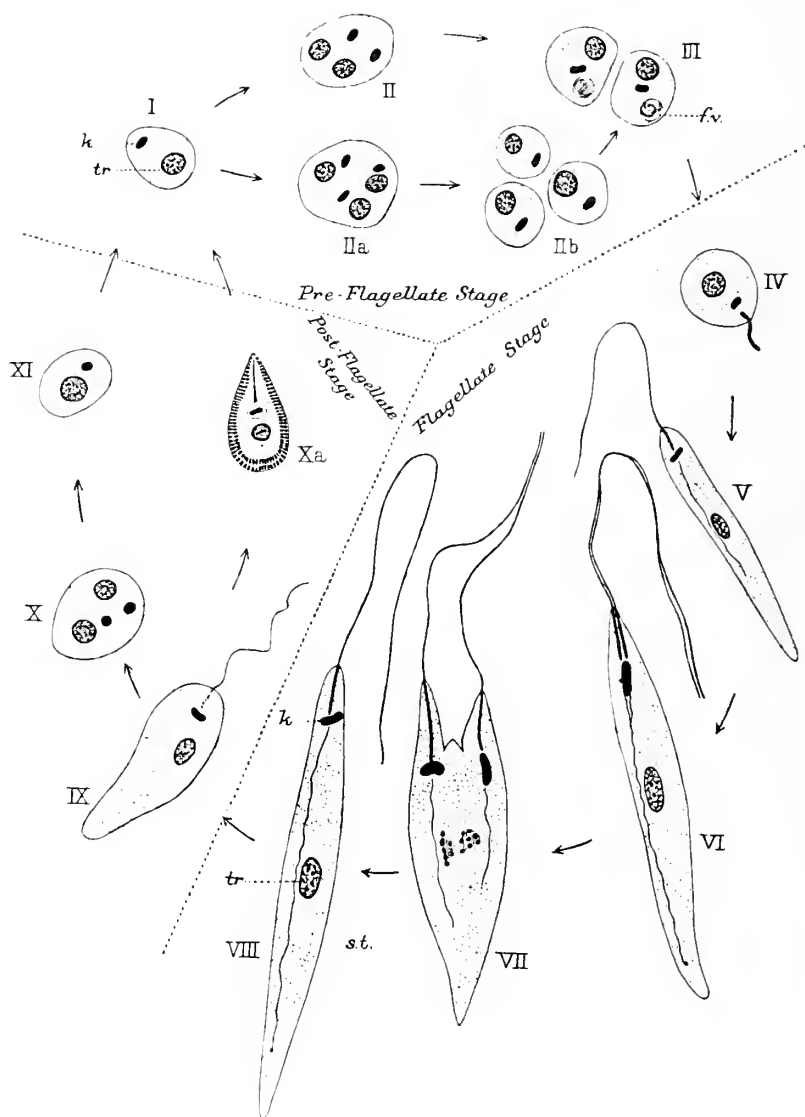


Diagram of the life-cycle of *Herpetomonas muscæ-domesticae* Burnett. Arrangement chiefly after Patton; figures after Léger, Patton, and Prowazek. I-III. Preflagellate stage. IV-VIII. Flagellate stage: V. Young flagellate. VI. Flagellate beginning to divide, flagellum having already divided. VII. Advanced stage of division. VIII. Adult flagellate. IX-XI. Post-flagellate stage: IX. Degeneration of flagellum. Xa. Post-flagellate stage completed by formation of gelatinous covering, containing double row of granular bodies (Prowazek). f.v. Flagellar vacuole. k. Kinetonucleus. s.t. Spiral chromophilous thread. tr. Trophonucleus.

parasitic in the gut of *Nepa cinerea*, from which figures it may be understood how the mistake has arisen. Through this misinterpretation Prowazek was led to consider that the parasite was of a bipolar type, in which the body had been doubled on itself so that the two ends came together and the flagellum remained distinct. The flagellum, according to Léger, is continued into the cytoplasm as a thin thread, which stains with difficulty, and terminates in a double granule above the kinetonucleus; this double granule is no doubt the "diplosome" of Prowazek. According to the latter author another deeply staining double thread (*s.t.*), that appears to be spirally coiled, runs backwards from the kinetonucleus and terminates posteriorly in a distinct granule, shown in fig. VIII.

The flagellates congregate in the proventriculus or in the posterior region of the intestine, where they become united by their anterior ends to form rosettes. Prowazek states that in the rosette condition the living portion of the flagellate resides, as it were, in the long tail-like process.

Patton divides the life-cycle of *H. muscæ-domesticæ* into three stages—the preflagellate, flagellate, and post-flagellate. The last two are common, but the first stage is not common, and Prowazek appears to have overlooked it. For convenience I have described the flagellate stage first, and the process of division in this stage is simple longitudinal fusion. The nuclei divide independently, and the kinetonucleus usually precedes the trophonucleus. The latter undergoes a primitive type of mitosis, in which Prowazek recognised eight chromosomes (VII). The flagellum divides longitudinally, and each of the two halves of the kinetonucleus appropriates one of the halves with its basal granule.

The preflagellate stage, which Patton (1909) describes, usually occurs in the masses which lie within the peritrophic membrane.¹ They are round or slightly oval bodies (I), their average breadth being 5.5μ . The protoplasm is granular and

¹ I assume that Patton refers to this membrane by the term "peritricheal membrane."

contains a trophonucleus and kinetonucleus. Division takes place by simple longitudinal division or multiple segmentation, and in this manner a large number of individuals are formed (II *b* and III). These develop into the flagellate stage: a vacuole, the flagellar vacuole (III, *f.v.*) appears between the kinetonucleus and the rounded end of the pre-flagellate form, and in it the flagellum appears as a single coiled thread, which is extended when the vacuole has approached the surface.

The flagellate form has already been described, and in the concluding portion of the flagellate stage, which, according to Prowazek, is found in starved flies, these forms are found collecting in the rectal region, and attaching themselves by their flagellar ends in rows to gut epithelium. The more external ones begin to shorten, during which process the flagella degenerate (IX) and are shed. Thus a palisade of parasites is formed, the outer ones being rounded and devoid of flagella, and some of them may be found dividing (X). Léger (1902) terms these the "formes grégariennes," and maintains that the existence of these "gregarine" forms is a powerful argument in favour of the flagellate origin of the Sporozoa, which he had previously suggested, and which Bütschli had put forward in 1884. After the degeneration of the flagellum a thickened gelatinous covering is formed, containing a double row of granular bodies (X*a*), and these cysts are regarded by Patten as the post-flagellate stage. They pass out with the fæces, and dropping on the moist window-pane or on food, are taken up by the proboscides of other flies.

Prowazek describes dimorphic forms of the flagellate stage, which he regards as sexually differentiated forms, but Patton, in a letter to me, says that he is unable to find any of these complicated sexual stages. According to Prowazek, one of these forms is slightly larger than the other, and has a greater affinity for stain. The dimorphic forms conjugate; their cell substance and nuclei fuse, and a resting-stage cyst is formed, but the subsequent stages have not been followed. He further states that the sexually differentiated forms may force

their way into the ovaries, where they undergo autogamy and infect the subsequent brood.

In Madras Patton found that 100 per cent. of the flies were infected with the flagellate; Prowazek found it in 8 per cent. of the flies at Rovigno. In the cold season in the plains (India) Lingard and Jennings (l. c.) found the flagellate in less than 1 per cent. of the flies examined; in the hills (Himalayas), at an elevation of 7500 feet, the flagellates were most numerous during the hottest season of the year, and gradually decreased in number to October and November, when none were discovered.

One of the chief points of interest in connection with this flagellate is its similarity to the "Leishmann-Donovan" body, the parasite of kala-azar, as it was this resemblance that prompted Rogers (1905) to suggest that the latter parasite was a *Herpetomonas*, which I think Patton has now conclusively proved to be the case, and he calls it *Herpetomonas donovani* (Laveran and Mesnil).

Crithidia Muscæ-domesticæ Werner.

This parasite has been recently described by Werner (1908), who found it in the alimentary tracts of four out of eighty-two flies. It measures 10–13 μ in length, the length of the body being 5–7 μ and the flagellum 5–6 μ . As in other members of the genus *Crithidia*, which is closely allied to *Herpetomonas*, the breadth of the body is great compared with the length, and the kinetonucleus and trophonucleus are rather close together. A short, staining, rod-like body lies between the kinetonucleus and the base of the flagellum. The flagellum is single. Dividing forms undergoing longitudinal division were frequently found. The kinetonucleus appears to divide first, followed in succession by the flagellum and the trophonucleus. Forms undergoing division and showing a single trophonucleus and double kinetonucleus and flagellum were also found. Cases occurred in which the fission began at the

non-flagellate end of the body. No conjugating forms were found, nor any wandering into the ovaries.

Lingard and Jennings (l. c.) describe certain flagellates of a flag-shaped or rhomboidal nature, which I am strongly of the opinion are species of *Crithidia* and not species of *Herpetomonas*. Closely following Prowazek's account of *H. muscæ-domesticæ* they describe and figure all their forms as having two flagellæ in the flagellate stage. If one allows for the rupture of the flagellum from the bodies of the organism in making the film, some of their figures are not unlike those of *Crithidia gerriidis*, parasitic in the alimentary tract of an Indian water-bug, *Gerris fossarum* Fabr., and described by Patton (1908).

2. Nematoda—*Habronema muscæ* (Carter).

Carter (1861) appears to be the first to have described a parasitic worm in *M. domestica*. He described a bisexual nematode infesting this insect in Bombay, and found that: "Every third fly contains from two to twenty or more of these worms, which are chiefly congregated in, and confined to, the proboscis, though occasionally found among the soft tissues of the head and posterior part of the abdomen." His description of this nematode, to which he gave the name *Filaria muscæ*, is as follows: "Linear, cylindrical, faintly striated transversely, gradually diminishing towards the head, which is obtuse and furnished with four papillæ at a little distance from the mouth, two above and two below; diminishing also towards the tail, which is short and terminated by a dilated round extremity covered with short spines. Mouth in the centre of the anterior extremity. Anal orifice at the root of the tail." He gives the length as being one eleventh of an inch and the breadth as one three hundred and thirteenth of an inch. In his description of his figures of the worm he calls what is evidently the anterior region of the intestine the "liver." Von Linstow (1875) described a small nematode, which he calls *Filaria stomoxeos*, from the

head of *S. calcitrans*; this larva measured 1.6 to 2 mm. in length. Generali (1886) described a nematode from the common fly, which he calls *Nematodum spec.* It is highly probable, as my friend Dr. A. E. Shipley has suggested to me, that Generali's nematode and the *F. muscæ* of Carter are identical. Diesing (1861) created the genus *Habronema* for the *Filaria muscæ* of Carter, and his description is practically a translation of Carter's original description. Piana (1896) describes a nematode from the proboscis of *M. domestica*, which, in the occurrence of the male and female genital organs in the same individual, he says, resembles Carter's nematode. He finds that at certain seasons of the year and in certain localities it is very rare, while at others it may occur in 20-30 per cent. of the flies. The larva, after fixation, measured 2.68 mm. in length and 0.08 mm. in breadth. It was cylindrical and gently tapering off at the extremities, with the mouth terminal.

Out of the many hundreds of flies which I have dissected I have only found two specimens of this nematode (fig. 18). From the descriptions given by Carter and Piana and the figures of the latter I feel convinced that their specimens and mine are the same species, called by Diesing *Habronema muscæ* (Carter). It is linear, cylindrical, tapering gradually towards both ends. The anterior end is slightly rounded, having the mouth in the centre. I am unable to confirm the presence of the four papillæ which Carter describes as a little distance from the mouth, nor are they figured by Piana. The cuticle is very faintly marked with transverse striations. The common genital and anal orifice is situated at a short distance from the posterior end of the body, which tapers off slightly more than the anterior end and terminates in a small dilated extremity, which is covered with minute spines (fig. 19). My specimens appear to be immature adult forms, not having reached sexual maturity. The species measures 2 mm. in length and 0.04 mm. in breadth. The specimens that I obtained were situated in the head region, between the optic ganglia and the cephalic air-sacs, from which position they

could easily move down into the cavity of the proboscis. I am unaware of any previous record of the occurrence of *Habronema muscæ* in this country, but I have no doubt that if one searched specially for it it would be found to occur more commonly than might appear from my experience, and to be generally distributed with its host throughout the world.

The occurrence of a parasitic worm in this position is of great interest, even though *M. domestica* is not a blood-sucking species and the nematode is not of the nature of *Filaria bancrofti*. There is no reason, however, why *M. domestica* should not under certain conditions carry pathogenic nematodes, which might easily get on to the food of man.

3. Dissemination of Parasitic Worms.

In this connection reference might be made to the experiments of Grassi (1883) to which reference is made by Nuttall in his valuable memoir (1899). Grassi broke up segments of *Tænia solium* in water; they had previously been preserved in alcohol for some time. Flies sucked up the eggs in the water and he found them unaltered in the fæces. *Oxyuris* eggs were also passed unaltered. In another experiment flies fed on the eggs of *Trichocephalus* and he found the eggs some hours afterwards in the flies' fæces, which had been deposited in the story beneath the laboratory; he also caught flies in this kitchen with their intestines full of eggs.

Calandruccio¹ examined flies (? species) which had settled upon fæces containing the ova of *Tænia nana*. The ova were found in the flies' intestines. The excrement deposited by a fly on sugar contained two or three ova of the *Tænia*. By means of such infected sugar a girl was infected, and ova of *T. nana* were found in her stools on the twenty-seventh day.

¹ "Ulteriori ricerche sulla *Tænia nana*," 'Boll. Soc. Zool. Ital. Roma,' vol. vii, pp. 65-69; also in 'Boll. Acad. Gioenia, Catania,' Fasc. 89, pp. 15-19.

Nuttall (l. c.) records a personal communication of Stiles, who placed the larvæ of *Musca* with female *Ascaris lumbricoides*, which they devoured together with the eggs contained by the nematodes. The larvæ and adult flies contained the eggs of the *Ascaris*, and as the weather at the time of the experiment was very hot the *Ascaris* eggs developed rapidly and were found in different stages of development in the insect, thus proving, as Nuttall points out, "that the latter may serve as disseminators of the parasite." These experiments of Grassi and Stiles show that flies can act as carriers of the eggs of these parasitic worms, and that man could be infected by the fly depositing its excreta on his food, or being accidentally immersed in food as flies frequently are.

VII. THE DISSEMINATION OF PATHOGENIC ORGANISMS BY *MUSCA DOMESTICA* AND ITS NON-BLOOD-SUCKING ALLIES.

Although *M. domestica* is unable to act as a carrier of pathogenic micro-organisms in a manner similar to that of the mosquito, so far as we know at present, nevertheless its habits render it a very potent factor in the dissemination of disease by the mechanical transference of the disease germs. These habits are the constant frequenting and liking for substances used by man for food on the one hand and excremental products, purulent discharges, and moist surfaces on the other. Should these last contain pathogenic bacilli, the proboscis, body, and legs of the fly are so densely setaceous (see fig. 20) that a great opportunity occurs, with a maximum amount of probability, for the transference of the organisms from the infected material to either articles of food or such moist places as the lips, eyes, etc. As I have already pointed out (1907), *M. domestica* is unable to pierce the skin, as certain persons have suggested. The structure of the proboscis will not permit the slightest piercing or pricking action, which fact eliminates such an inoculative method of infection. It is as a mechanical carrier, briefly, that *M.*

domestica and such allies as *H. canicularis*, etc., though to a less degree, may be responsible for the spread of infectious disease of a bacillary nature, and an account will now be given of the rôle which this insect plays in the dissemination of certain diseases.¹ Before doing so, however, it should be pointed out that whereas in some of the diseases the epidemiological evidence adduced in support of the transference of disease germs by flies is confirmed bacteriologically, in others only the former evidence exists. Should neither form of evidence be available in support of the idea that *M. domestica* plays a part in the dissemination of the infection of a particular disease, it is essential, nevertheless, that if such a method of transference is possible the potency of this insect should be realised. This potency is governed by such factors as the presence of *M. domestica*; its access to the infected or infective material, this being attractive to the insect either because it is moist or because it will serve as food for itself or its progeny; and a certain power of resistance for a short time against desiccation on the part of the pathogenic organisms, although, as in the case of the typhoid bacillus, the absence of this factor is not fatal to the idea, as it may be overcome by the fact that the fly is able to take on its appendages an amount sufficient to resist desiccation for a short time. The last factor is the presence of suitable culture media, such as certain foods, or moist surfaces as the mouth, eyes, or wounds, for the reception of the organisms which have been carried on the body or appendages of the fly. If these conditions are satisfied the possibility of *M. domestica* or its allies playing a part in the transference of the infection should be carefully considered, and this suggestive evidence will be discussed in certain of the diseases which follow, in addition to the epidemiological and bacteriological evidence.

¹ Though it should be unnecessary, I wish to explain, as I have been occasionally misunderstood by medical men and others, that *M. domestica* is not regarded as being the cause of any disease, but as a carrier of the infection.

1. Typhoid Fever.

Of all infectious diseases the conditions in this are most favourable for the transference of infection by *M. domestica*, and it is no doubt on this account that the greatest attention has been paid to the rôle of house-flies in the dissemination of this disease. The chief favourable condition is that the typhoid bacillus occurs in the stools of typhoid and incipient typhoid cases. Human excrement attracts flies not only on account of its moisture but as suitable food for the larvæ. The infected excrement is often accessible to flies, especially in military camps, as will be shown shortly, and the flies also frequent articles of food and not infrequently the moist lips of man. Such are the conditions most suitable for the transference of the bacilli, and it is on account of the frequent coincidence of these conditions that flies can play, and have played, such an important rôle in the dissemination of this disease among communities, in spite of the fact that the typhoid bacillus cannot survive desiccation, which I think is an argument against its being carried by dust.

Epidemiological and other evidence.—There is a very large amount of testimony given as to the rôle played by flies in the spread of enteric in military stations and camps, and especially during the two wars—the Spanish-American and the Boer War. All the conditions most favourable for the dissemination of the bacilli by flies were, and in many military stations are still, present; open latrines or filth-trenches accessible to flies on the one hand and on the other the men's food within a short distance of the latrines. I cannot do better than repeat the evidence in the words of the witnesses and allow it to speak for itself.

Vaughan, a member of the U.S. Army Typhoid Commission of 1898, states:¹

“My reasons for believing that flies were active in the dissemination of typhoid fever may be stated as follows:

¹ In a paper, “Conclusions Reached after a Study of Typhoid Fever among American Soldiers,” read before the American Medical Association at Atlantic City, N.J., in 1900.

“(a) Flies swarmed over infected faecal matter in the pits and then visited and fed upon the food prepared for the soldiers in the mess-tents. In some instances where lime had recently been sprinkled over the contents of the pits, flies with their feet whitened with lime were seen walking over the food.

“(b) Officers whose mess-tents were protected by screens suffered proportionately less from typhoid fever than did those whose tents were not so protected.

“(c) Typhoid fever gradually disappeared in the fall of 1898 with the approach of cold weather and the consequent disabling of the fly.

“It is possible for the fly to carry the typhoid bacillus in two ways. In the first place faecal matter containing the typhoid germs may adhere to the fly and be mechanically transported. In the second place, it is possible that the typhoid bacillus may be carried in the digestive organs of the fly and may be deposited with its excrement.”

One of his conclusions was that infected water was not an important factor in the dissemination of typhoid in the national encampments of 1898, since only about one fifth of the soldiers in the national encampments during the summer of that year developed typhoid fever, whereas about 80 per cent. of the total deaths were due to this disease. In the latter connection Sternberg (1899) refers to a report of Dr. Reed upon an epidemic in the Cuban War, in which it was stated that the epidemic was clearly not due to water infection but was transferred from the infected stools of the patients to the food by means of flies, the conditions being especially favourable for this means of dissemination. Sternberg, as Surgeon-General of the U.S. Army, issued the following instructions¹: “Sinks should be dug before a camp is occupied or as soon after as practicable. The surface of the faecal matter should be covered with fresh earth or quicklime or ashes three times a day.” I think that the instructions of that ancient leader of men, Moses, who probably had

¹ ‘Circular No. 1 of the Surgeon-General of the U.S. Army,’ April, 1898.

experienced the effects of flies, were even better than these. He said (Deut., Ch. xxiii, v. 12-13): "Thou shalt have a place also without the camp whither thou shalt go forth abroad; and thou shalt have a paddle [or 'shovel'] among thy weapons; and it shall be, when thou sittest down abroad, thou shalt dig therewith, and shalt turn back and cover that which cometh from thee."

Sternberg is of the opinion that typhoid fever and camp diarrhœa are frequently communicated to soldiers through the agency of flies, "which swarm about fæcal matter and filth of all kinds deposited upon the ground or in shallow pits, and directly convey infectious material attached to their feet or contained in their excreta to the food which is exposed while being prepared in the common kitchen, or while being served in the mess-tent."

Veeder (1898), in referring to the conditions existing in the camps of the Spanish-American war, says that in the latrine trenches he saw "fæcal matter fresh from the bowel and in its most dangerous condition, covered with myriads of flies, and at a short distance there was a tent, equally open to the air, for dining and cooking. To say that the flies were busy travelling back and from between these two places is putting it mildly." Further, he says, "There is no doubt that air and sunlight kill infection, if given time, but their very access gives opportunity for the flies to do serious mischief as conveyers of fresh infection wherever they put their feet. In a very few minutes they may load themselves with the dejections from a typhoid or dysenteric patient, not as yet sick enough to be in hospital or under observation, and carry the poison so taken up into the very midst of the food and water ready for use at the next meal. There is no long and round-about process involved. It is very plain and direct. Yet when the thousands of lives are at stake in this way the danger passes unnoticed, and the consequences are disastrous and seem mysterious until attention is directed to the point; then it becomes simple enough in all conscience."

The Commission which investigated the outbreaks of

enteric fever that occurred in 1898 in the United States during this war came to the conclusion that "flies undoubtedly served as carriers of the infection" under the conditions which have already been described. Many other authorities bear witness to the same facts.

In our own South African war, a year or two later, the same conditions existed, and there was a very heavy loss of life from enteric fever. Writing on the subject, Dunne (1902) says: "The plague of flies which was present during the epidemic of enteric at Bloemfontein in 1900 left a deep impression on my mind, and, as far as I can ascertain from published reports, on all who had experience on that occasion. Nothing was more noticeable than the fall in the admissions from enteric fever coincident with the killing off of the flies on the advent of the cold nights of May and June. In July, when I had occasion to visit Bloemfontein, the hospitals there were half empty, and had practically become convalescent camps." A similar experience is related by Tooth (1901). Referring to the rôle of flies he says: "As may be expected, the conditions in these large camps were particularly favourable to the growth and multiplication of flies, which soon became terrible pests. I was told by a resident in Bloemfontein that these insects were by no means a serious plague in ordinary times, but that they came with the army. It would be more correct to say that the normal number of flies was increased owing to the large quantities of refuse upon which they could feed and multiply. They were all over our food, and the roofs of our tents were at times black with them. It is not unreasonable to look upon flies as a very possible agency in the spreading of the disease, not only abroad but at home. It is a well-known fact that with the first appearance of the frost enteric fever almost rapidly disappears. . . . It seems hardly credible that the almost sudden cessation of an epidemic can be due to the effect of cold upon the enteric bacilli only. But there can be no doubt in the mind of anybody who has been living on the open veldt, as we have for three or four months, that flies are ex-

tremely sensitive to the change of temperature, and that the cold nights kill them off rapidly." In the discussion on this paper Church stated that "many nurses told me that if one went into a tent or ward in which the patients were suffering from a variety of diseases, one could tell at once which were the typhoid patients by the way in which the flies clustered about their mouths and eyes while in bed." It was further stated in the discussion that where the Americans used quicklime in their latrines the cooks in the neighbouring kitchens found that the food became covered with quicklime from the flies which came from the latrines to the kitchens.

Dr. Tooth, in a letter to me, says: "I am afraid my written remarks hardly express strongly enough the importance that I attach to flies as a medium of spreading infection. Of course I do not wish to under-rate the water side of the question, but once get, by that means, enteric into a camp the flies, in my opinion, are quite capable of converting a sporadic incidence into an epidemic. A pure water supply is an obvious necessity, but the prompt destruction of refuse of every description is every bit as important."

Smith (1903), in speaking of his experiences in South Africa, says that: "On visiting a deserted camp during the recent campaign it was common to find half a dozen or so open latrines containing a foetid mass of excreta and maggots." Similar observations were made by Austen (1904), who, describing a latrine that had been left a short time undisturbed, says: "A buzzing swarm of flies would suddenly arise from it with a noise faintly suggestive of the bursting of a percussion shrapnel shell. The latrine was certainly not more than one hundred yards from the nearest tents, if so much, and at meal-times men's mess-tins, etc., were always invaded by flies. A tin of jam incautiously left open for a few minutes became a seething mass of flies (chiefly *Pycnosoma chloropyga* Wied), completely covering the contents."

Howard (1900) referring to an American camp, where no effort was made to cover the faeces in the latrines, says: "The camp contained about 1200 men, and flies were extremely

numerous in and around the sinks. Eggs of *Musca domestica* were seen in large clusters on the *fæces*, and in some instances the patches were two inches wide and half an inch in depth, resembling little patches of lime. Some of the sinks were in a very dirty condition and had a very disagreeable odour."

A few examples of the prevalence of conditions favouring the dissemination of enteric by flies in permanent camps may be noted. Cockerill (1905), in describing camp conditions in Bermuda, mentions kitchens within one hundred yards of the latrines; the shallow privy, seldom or never cleaned out, and middens are found which contain masses of filth swarming with flies. He states that in more recent years the period of greatest incidence is in the summer, being chiefly due to flies and contaminated dust. Quill (1900), reporting on an outbreak of enteric in the Boer camp in Ceylon, states: "During the whole period that enteric fever was rife in the Boer camp flies in that camp amounted to almost a plague, the military camp being similarly infested, though to a less extent. The outbreak in the Boer camp preceded that among the troops; the two camps were adjacent, and the migration of the flies from the one to the other easy." Weir, reporting on an outbreak of enteric fever in the barracks at Umbala, India,¹ says that most of the pans in the latrines were half or quite full, and flies were very numerous in them and on the seats, which latter were soiled by the excreta conveyed by the flies' legs. The men stated that the plague of flies was so great that in the morning they could hardly go to the latrines. He found that the flies were carried from the latrines to the barrack-rooms on the clothes of the men. This state of affairs suggests another mode of infection, namely, *per rectum*. As Smith has pointed out (*l.c.*) it is not improbable that flies under these conditions may be inoculators of dysentery.

Aldridge (1907) gives some interesting statistics showing the influence of the presence of breeding-places of flies. Flies are found in greater numbers in mounted regiments than in

¹ 'Army Medical Department Report,' 1902, p. 207.

infantry, and he shows how this affects the incidence of enteric fever. In the British Army in India, 1902-05, the ratios per 1000 per annum of cases admitted were: cavalry 41.1, and infantry 15.5; and in the U.S. Army were: cavalry 5.74, and infantry 4.75. He states that: "A study of the incidence of enteric fever shows that stations where there are no filth trenches, or where they are a considerable distance from the barracks, all have an admission-rate below the average, and all but one less than half the average."

All these facts are equally applicable to the conditions in our own towns and cities. Where the old conservancy methods are used, such as pails and privy middens, the incidence of typhoid fever is greater than in those places where the system of water disposal has been adopted. I have examined the annual reports of the medical officers of health of several large towns where such conversions are being made, and they show a falling-off of the typhoid fever-rate coincident with this change. In Nottingham, for example,¹ in the ten years 1887-1896, there was one case of typhoid fever for every 120 houses that had pail-closets, one case for every 37 houses with privy middens, and one case for every 5.58 houses with water-closets. The last were scattered, and not confined to the prosperous districts of the town.

One of the most important investigations on the relation of flies to intestinal disease was that of Jackson (1907). He investigated the sanitary condition of New York harbour and found that in many places sewer outfalls had not been carried below low-water mark, consequently solid matter from the sewers was exposed on the shores, and that during the summer months on and near the majority of the docks in the city a large amount of human excreta was deposited. This was found to be covered with flies. The report, considered as a mere catalogue, is a most severe indictment against the insanitary condition of this great water front. By means of spot-maps he shows that the cases of typhoid are thickest

¹ "Typhoid Fever and the Pail System at Nottingham," *Lancet*, November 29th, 1902, p. 1489.

near the points found to be most insanitary. He shows, as English investigators have also shown, how the curves of fatal cases correspond with the temperature curves and with the curves of the activity and prevalence of flies which were obtained by actual counts. He also adduced bacteriological evidence, and it is stated that one fly was found to be carrying over one hundred thousand faecal bacteria.

Bacteriological evidence.—In addition to the evidence of Jackson, to which reference has been made, further proof that flies are able to carry the typhoid bacillus has been available for some years. Celli (1888) recovered the *Bacillus typhi abdominalis* from the dejections of flies which had been fed on cultures of the same, and he was able to prove that they passed through the alimentary tract in a virulent state by subsequent inoculation experiments. Ficker (1903) found that when flies were fed upon typhoid cultures they could contaminate objects upon which they rested. The typhoid bacilli were present in the head and on the wings and legs of the fly five days after feeding, and in the alimentary tract nine days after. Firth and Horrocks (1902), in their experiments, took a small dish containing a rich emulsion in sugar made from a twenty-four-hour agar slope of *Bacillus typhosus* recently obtained from an enteric stool and rubbed up with fine soil. This was introduced with some infected honey into a cage of flies together with sterile litmus agar plates and dishes containing sterile broth, which were placed at a short distance from the infected soil and honey. Flies were seen to settle on the infected matter and on the agar and broth. The agar plates and broth were removed after a few days, and after incubation at 37° C. for twenty-four hours colonies of *Bacillus typhosus* were found on the agar plates and the bacillus was recovered from the broth. In a further experiment the infected material was dusted over with fine earth to represent superficially buried dejecta, and the bacillus was isolated from agar plates upon which the flies had subsequently walked, as in the former experiment. They also found the bacillus on the heads, wings,

legs and bodies of flies which had been allowed to have access to infected material. Hamilton (1903) recovered *Bacillus typhosus* five times in eighteen experiments from flies caught in two undrained privies, on the fences of two yards, on the walls of two houses and in the room of an enteric fever patient. A series of careful experiments were made by Sellars¹ in connection with Niven's investigations on the relation of flies to infantile diarrhoea. Out of thirty-one batches of house-flies carefully collected in sterilised traps in several thickly populated districts in Manchester he found, as a result of cultural and inoculatory experiments, that bacteria having microscopical and cultural characters resembling those of the *Bacillus coli* group were present in four instances, but they did not belong to the same kind or variety. Buchanan (1907) was unable to recover the bacilli from flies taken from the enteric ward of the Glasgow Fever Hospital. Flies were allowed to walk over a film of typhoid stool and then transferred to the medium (Grünbaum and Hume's modification of MacConkey's medium), and subsequently allowed to walk over a second and a third film of medium. Few typhoid bacilli were recovered and none from the second and third films. Sangree (1899) performed somewhat similar experiments to those of Buchanan and recovered various bacilli in the tracks of the flies. This method of transferring the flies immediately from the infected material to the culture plate is not very satisfactory, as I have already pointed out (1908), as it would be necessary for the flies to be very peculiarly constructed not to carry the bacilli. The fly should be allowed some freedom before it has access to the medium to simulate natural conditions. Experiments of this kind were carried out in the summer of 1907 by Dr. M. B. Arnold (superintendent of the Manchester Fever Hospital) and myself. Flies were allowed to walk over a film of typhoid stool and then were transferred to a wire cage, where they remained for twenty-four hours with the opportunity

¹ Recorded in the 'Report on the Health of the City of Manchester, 1906,' by James Niven, pp. 86-96.

of cleaning themselves, after which they were allowed to walk over the films of media. Although we were unable to recover *B. typhosus* the presence of *B. coli* was demonstrated. *B. coli* was also obtained from flies obtained on a public tip upon which the contents of pail-closets had been emptied; the presence of *B. coli*, however, may not necessarily indicate recent contamination with human excrement. Aldridge (l. c.) isolated a bacillus apparently belonging to the paratyphoid group from flies caught in a barrack latrine in India during an outbreak of enteric fever. In appearance and behaviour to tests it was very similar to *B. typhosus*.

Although we are not certain yet as to the specific organism or organisms which cause the intestinal disease known as infantile or summer diarrhœa, which is so prevalent during the summer months and is responsible for so great a mortality among young children, I think we must consider the relationship of *M. domestica* and its ally *Homalomyia canicularis* to this disease epidemiologically similar to typhoid fever.

2. Anthrax.

In considering the relation of flies to anthrax several facts should be borne in mind. As early as the eighteenth century it was believed that anthrax might result from the bite of a fly, and the idea has been used by Murger in his romance 'Le Sabot Rouge.' A very complete historical account of this is given by Nuttall (1899). Most of the instances in support of this belief, however, that flies may carry the infection of anthrax, refer to biting flies. As I have already pointed out, *M. domestica* and such of its allies as *H. canicularis*, *C. erythrocephala*, *C. vomitoria*, and *Lucilia cæsar* are not biting or blood-sucking flies. The nearest allies of *M. domestica* which suck blood in England are *S. calcitrans*, *Hæmatobia stimulans* Meigen, and *Lyperosia irritans* L.; the rest of the blood-sucking flies which may be considered in this connection belong to the family Tabanidæ, including the common genera *Hæma-*

lopota, *Tabanus*, and *Chrysops*. These biting and blood-sucking flies live upon the blood of living rather than dead animals. But it is from the carcasses and skins of animals which have died of anthrax that infection is more likely to be obtained, and I believe that such flies as the blow-flies (*Calliphora* spp.), and sometimes *M. domestica* and *Lucilia cæsar*, which frequent flesh and the bodies of dead animals for the purpose of depositing their eggs and for the sake of the juices, are more likely to be concerned in the carriage of the anthrax bacillus and the causation of malignant pustule than are the blood-sucking flies. Consequently, as *M. domestica* and its allies only are under consideration, and for the sake of brevity, the relation to anthrax of the non-biting flies only will be considered here.

The earliest bacteriological evidence in support of this belief was published by Raimbert (1869). He experimentally proved that the house-fly and the meat-fly were able to carry the anthrax bacillus, which he found on their probosces and legs. In one experiment two meat-flies were placed from twelve to twenty-four hours in a bell-jar with a dish of dried anthrax blood. One guinea-pig was inoculated with a proboscis, two wings and four legs of a fly, and another with a wing and two legs. Both were dead at the end of sixty hours, anthrax bacilli being found in their blood, spleen, and heart. He concludes: "Les mouches qui se posent sur les cadavres des animaux morts du Charbon sur les dépouilles, et s'en nourrissent, ont la faculté de transporter les virus charbonneux déposé sur la peau peut en traverser les différentes couches." Davaine (1870) also carried out similar experiments with *C. vomitoria*, which was able to carry the anthrax bacillus. Bollinger (1874) found the bacilli in the alimentary tract of flies that he had caught on the carcass of a cow dead of anthrax. Buchanan (l. c.) placed *C. vomitoria* under a bell-jar with the carcass of a guinea-pig (deprived of skin and viscera) which had died of anthrax. He then transferred them to agar medium and a second agar capsule, both of which subsequently showed a profuse growth

of *B. anthracis* as one might expect. Specimens of *M. domestica* were also given access to the carcass of an ox which had died of anthrax; they all subsequently caused growths of the anthrax bacillus on agar. I entirely agree with Nuttall, who says: "It does seem high time, though, after nearly a century and a half of discussion, to see what would be the result of properly carried out experiments. That ordinary flies (*M. domestica* and the like) may carry about and deposit the bacillus of anthrax in their excrements, or cause infection through their soiled exterior coming in contact with wounded surfaces or food, may be accepted as proven in view of the experimental evidence already presented."

3. Cholera.

One of the first to suggest that flies may disseminate the cholera spirillum was Nicholas (1873), who, in an interesting and prophetic letter, said: "In 1849, on an occasion of going through the wards of the Malta Hospital, where a large amount of Asiatic cholera was under treatment, my first impression of the possibility of the transfer of the disease by flies was derived from the observation of the manner in which these voracious creatures, present in great numbers, and having equal access to the dejections and food of the patients, gorged themselves indiscriminately, and then disgorged themselves on the food and drinking utensils. In 1850 the 'Superb,' in common with the rest of the Mediterranean squadron, was at sea for nearly six months; during the greater part of the time she had cholera on board. On putting to sea the flies were in great force, but after a time the flies gradually disappeared and the epidemic slowly subsided. On going into Malta Harbour, but without communicating with the shore, the flies returned in greater force, and the cholera also with increased violence. After more cruising at sea the flies disappeared gradually, with the subsidence of the disease. In the cholera years of 1854 and 1866 in this country the periods of occurrence and disappear-

ance of the epidemics were coincident with the fly-season." Buchanan (1897), in a description of a gaol epidemic of cholera which occurred at Burdwan in June, 1896, states that swarms of flies occurred about the prison, outside which there were a number of huts containing cholera cases. Numbers of flies were blown from the sides where the huts lay into the prison enclosure, where they settled on the food of the prisoners. Only those prisoners who were fed in the gaol enclosure nearest the huts acquired cholera, the others remaining healthy.

Bacteriological evidence.—Maddox (1885) appears to have been the first to conduct experiments with a view to demonstrating the ability of flies to carry the cholera spirillum, or, as it was then called, the "comma-bacillus." He fed the flies *C. vomitoria* and *Eristalis tenax* (the "drone-fly") on pure and impure cultures of the spirillum, and appears to have found the motile spirillum in the fæces of the flies. He concludes that these insects may act as disseminators of cholera. During a cholera epidemic Tizzoni and Cattani (1886) showed experimentally that flies were able to carry the "comma-bacillus" on their feet. They also obtained, in two out of three experiments, the spirillum from cultures made with flies from one of the cholera wards. Sawtchenko (1892) made a number of careful experiments. Flies were fed on bouillon culture of the cholera spirillum, and to be certain that the subsequent results should not be vitiated by the presence of the spirillum on the exterior of the flies, he disinfected them externally and then dissected out the alimentary canal, with which he made cultures. In the case of flies which had lived for forty-eight hours after feeding, the second and third cultures represented pure cultures of the cholera spirillum. Simmonds (1892) placed flies on a fresh cholera intestine, and afterwards confined them from five to forty-five minutes to a vessel in which they could fly about. Roll cultures were then made, and colonies of the cholera spirillum were obtained after forty-eight hours. Colonies were also obtained from a fly one and a half hours after having

access to a cholera intestine, and also from flies caught in a cholera post-mortem room. Uffelmann (1892) fed two flies on liquefied cultures of the cholera spirillum, and after keeping one of them for an hour in a glass he obtained 10,500 colonies from it by means of a roll culture; from the other, which was kept two hours under the glass, he obtained twenty-five colonies. In a further experiment he placed one of the two flies similarly infected with the spirillum in a glass of sterilised milk, which it was allowed to drink. The milk was then kept for sixteen hours at a temperature of 20–21° C., after which it was shaken, and cultures were made from it; one drop of milk yielded over one hundred colonies of the spirillum. The other fly was allowed to touch with its proboscis and feed upon a piece of juicy meat that was subsequently scraped. From one half of the surface twenty colonies, and from the other half one hundred colonies, of the spirillum were obtained. These experiments show the danger which may result if flies having access to a cholera patient, and bearing the spirillum, have access also to the food. Macrae (1894) records experiments in which boiled milk was exposed in different parts of the gaol at Gaya in India, where cholera and flies were prevalent. Not only did this milk become infected, but the milk placed in the cowsheds also became infected. The flies had access both to the cholera stools and to such food as rice and milk.

These foregoing experiments prove beyond doubt the ability of flies to carry the cholera spirillum, both internally and externally, in a virulent condition, and to infect food.

4. Tuberculosis.

Although it may be considered to be hardly necessary to introduce flies as a means of disseminating the tubercle bacillus, it has, nevertheless, been proved experimentally that they are able to carry the bacillus in a virulent condition. As early as 1887 Spillman and Haushalter carried on experiments in which they found the tubercle bacillus in large

numbers in the intestines of flies from a hospital ward, and also in the dejections which occurred on the windows and walls of the ward. Hoffmann (1886) also found tubercle bacilli in the excreta of flies in the room where a patient had died of tuberculosis, and he also found the bacilli in the intestinal contents. One out of three guinea-pigs which were inoculated with the intestines died; two inoculations with the excreta had no effect, which led him to believe that the bacilli became less virulent in passing through the alimentary tract. But Celli (l. c.) records experiments in which two rabbits inoculated with the excreta of flies fed with tubercular sputum developed the disease. Hayward (1904) obtained tubercle bacilli in ten out of sixteen cultures made from flies which had been caught feeding on bottles containing tuberculous sputum. Tubercle bacilli were also recovered from cultures made from the fæces of flies which had fed in the same manner, which apparently caused a kind of diarrhœa in the flies, and they died from two to three days afterwards. Fæces of flies fed on tubercular sputum were rubbed up in sterile water and injected into the peritoneal cavity of guinea-pigs, which developed tuberculosis. Buchanan (l. c.) allowed flies to walk over a film of tubercular sputum and then over agar; a guinea-pig died of tuberculosis in thirty-six days by inoculating it with the resulting culture.

5. Ophthalmia.

Flies have been suggested as playing an important part in the spread of conjunctivitis, especially Egyptian ophthalmia, and although, so far as I have been able to discover, we have no bacteriological evidence in favour of the belief, the circumstantial evidence is sufficiently strong to warrant it.

In speaking of its occurrence at Biskra, Laveran (1880) says that in the hot season the eyelids of the indigenous children are covered with flies, to the attentions of which they submit; in this way the infectious discharge is carried on the legs and probosces of flies to the healthy children.

Dr. Andrew Balfour, of the Gordon College, Khartoum, in a letter to me, says that the Koch-Weeks bacillus is generally recognised as being the exciting cause of Egyptian ophthalmia. He says, "Ophthalmia is not nearly so common in the Sudan as in Egypt, nor are flies so numerous; doubtless the two facts are associated." Dr. MacCallan, of the Egyptian Department of Public Health, in answer to my inquiries, says that acute ophthalmias are more liable to transmission by flies than is trachoma. In his opinion the spread of the latter is, to a comparatively small extent, through the agency of flies, but it is mainly effected by direct contact of the fingers, clothes, etc.

The Koch-Weeks bacillus was first seen by Koch (1883) in Egypt in cases of acute catarrhal ophthalmia. He found that two distinct diseases were referred to under the name; in the severe purulent form he found diplococci, which he identified as very probably *Gonococci*; in the more catarrhal form he found small bacilli in the pus-corpuseles. He ascribed the propagation of the disease to flies, which were often seen covering the faces of children. Axenfeld (1908) states that "almost the only organisms occurring in acute epidemics of catarrhal conjunctivitis are the Koch-Weeks bacillus (perhaps also influenza bacillus), and the pneumococcus (in Egypt the gonococcus also, rarely *subtilis*). Other pathogenic conjunctival organisms¹ only exceptionally occur." And, further, "*Gonococci* and Koch-Weeks bacilli evidently lose their power of causing a conjunctivitis very slowly indeed, and are very independent of any disposition." His statement that, "on account of their great virulence and the marked susceptibility to them, a very small number suffices," is important in considering the relation of flies to the spread of the disease, although, as he remarks, every infection does not produce the disease. The fact that the Koch-Weeks bacillus cannot resist dryness cannot be urged as an argument

¹ In this connection he states (p. 236): "We can make the general statement that the staphylococcus in the conjunctiva is not contagious."

against the spread of the infection by flies, or the same would apply to the typhoid bacillus, whose carriage by flies is proven. Axenfeld mentions L. Müller and Lakah and Khouri as advocating the view that flies may spread the infection more readily. In view of the fact that, as the same author states, "Koch-Weeks conjunctivitis is to be classed with the most contagious infectious disease which we know of," it is important that the rôle of flies should be fully recognised. Notwithstanding the occurrence in this country of flies in less numbers than in such countries as Egypt, it would be well to bear in mind the probable influence of flies in cases of acute conjunctivitis, such as those described by Stephenson (1897) in our own country. The sole difference between the disease in Egypt and here is, as Dr. Bishop Harman points out to me in a letter, that "the symptoms produced (in Egypt) are, from climate and dirtiness of the subjects, more severe, and that there is found a greater number of cases of gonorrhœal disease than in England"; and, I would add, a far greater number of flies. This disease is eminently suited for dissemination by flies, both on account of the accessibility of the infectious matter in the form of a purulent discharge from the eyes and on account of the flies' habit of frequenting the eyes.

6. Plague.

Although fleas are considered to be the chief agents in the dissemination of the plague bacillus in spite of the fact that the proof is not absolutely convincing, it is nevertheless interesting, and certainly not unimportant, to refer to the series of experiments of Nuttall (1897) on *M. domestica*. In these experiments he conclusively proved that flies were able to carry the plague bacillus, and that they subsequently died of the disease. Flies were fed upon the crushed organs of animals which had died of plague. Control flies were fed in a similar manner on the organs of uninfected animals, and the control experiments were kept under the same conditions.

In two of the experiments the flies were all dead on the seventh and eighth days respectively, at a temperature of 14° C. At higher temperatures he found that flies died more rapidly. He was able to show that the flies contained the bacilli in a virulent condition for about two days after they had fed on infected organs; this, and the fact that the infected flies can live for several days, are extremely important from the practical standpoint, as indicating that flies should neither be allowed to have access to the bodies or excreta of cases of plague, nor to the food.

7. Miscellaneous.

There are on record a number of suggestions that flies may be responsible for the dissemination of other diseases caused by bacteria and other micro-organisms, and some account will now be given of these and the experiments in support of such beliefs.

If flies have access to wounds of an inflammatory and suppurative nature they are liable to transport the *Staphylococci* to other spots. Buchanan (1907) allowed *M. domestica* to walk over a film of *Staphylococcus pyogenes aureus* from an abscess, and afterwards over agar; a mixed growth resulted, in which *S. pyogenes aureus* predominated. Celli (l.c.) records experiments which proved that *S. pyogenes aureus* retains its virulence after passing through the intestine of the fly.

In the experiments carried out in 1907 by my friend Dr. M. B. Arnold and myself, he chose *B. prodigiosus* for the purposes of the experiment, as it is easily recognisable and not likely to be accidentally introduced. Flies which had just emerged from the pupæ, and therefore not already contaminated with an extensive bacterial flora, were allowed to walk over a film of the bacillus, after which they were confined to sterile glass tubes. At varying periods they were taken out and allowed to walk over the culture plates. Those confined for over twelve hours retained the bacilli on their

TABLE SHOWING SOURCES OF BACTERIA FROM FLIES.

Date.	Source.	Total number of bacteria.	Total acid bacteria.	Rapid liquefying bacteria.	Slow liquefying bacteria.	<i>Bacterium lactis acid.</i> , Group A, Class 1.	<i>Coli aerogenes</i> , Group A, Class 2.
1907							
July 27	(a) 1 fly, bacteriological laboratory	3,150	250	600	100	—	—
" 27	(b) 1 fly, bacteriological laboratory	550	100	—	—	—	—
Aug. 6	(c) 19 cow-stable flies	7,980,000	220,000	—	20,000	—	—
	Average per fly	420,000	11,600	—	1,000	—	—
" 14	(d) 94 swill-barrel flies	155,000,000	8,950,000	—	—	4,320,000	4,630,000
	Average per fly	1,660,000	95,300	—	—	46,000	49,300
" 14	(e) 144 pig-pen flies	133,000,000	2,110,000	100,000	266,000	933,000	1,176,000
	Average per fly	923,000	18,700	700	1,150	6,500	12,000
Sept. 4	(f) 18 swill-barrel flies	118,800,000	40,480,000	—	14,500,000	10,480,000	30,000,000
	Average per fly	6,600,000	2,182,000	—	804,000	582,000	1,600,000
" 21	(g) 30 dwelling-house flies	1,425,000	125,000	—	12,500	—	—
	Average per fly	47,580	4,167	—	417	—	—
" 21	(h) 26 dwelling-house flies	22,880,000	22,596,000	120,000	34,000	—	—
	Average per fly	880,000	869,000	4,600	1,300	—	—
" 27	(i) 110 dwelling-house flies	35,500,000	13,670,000	8,840,000	125,000	—	—
	Average per fly	322,000	124,200	80,300	1,100	—	—
Aug. 20	(j) 1 large blue-bottle blowfly	308,700	(a)	—	—	—	—
	Total average of 414 flies	1,222,570	367,300	7,830	73,500	—	—
	Average per cent. of 414 flies	—	30	6	6	—	—
	Average per fly of 256 flies, experiments (d), (e), and (f)	3,061,000	765,000	230	268,700	211,500	553,800
	Average per cent. of 256 flies, experiments (d), (e), and (f)	—	25	—	8	7	18

(a) 2200 mould spores.

appendages and transferred them subsequently to the culture media, but they were not recovered from those flies which were kept in confinement for twenty-four hours; a large number of flies, however, were not used.

Dr. Kerr, of Morocco, in a paper on "Some Prevalent Diseases in Morocco," read before the Glasgow Medico-Chirurgical Society (December 7th, 1906), described epidemics of Syphilis where, according to the author, the disease was spread by flies which had been feeding upon the open sores of a syphilitic patient.

Howard (1909) calls attention to an important investigation carried on by Esten and Mason (1908) on the rôle which flies play in the carriage of bacteria to milk. The flies were caught by means of a sterile net; they were then introduced into a sterile bottle and shaken up in a known quantity of sterilised water to wash the bacteria from their bodies and to simulate the number of organisms that would come from a fly falling into a quantity of milk. They summarised their results in the table given on p. 403.

From that table it will be seen that the numbers of bacteria carried by a single fly may range from 550 to 6,600,000, while the average number was about 1,222,000. Commenting on these results, the authors state that "early in the fly-season the numbers of bacteria on flies are comparatively large. The place where flies live also determines largely the numbers that they carry." From these results the importance of keeping flies away from milk and other food will readily be seen.

VIII. FLIES AND INTESTINAL MYIASIS.

The larvæ of *M. domestica* and its allies are frequently the cause of intestinal myiasis and diarrhœa in children. The occurrence of the larvæ in the human alimentary tract may be accounted for in several ways. The flies may have deposited the eggs on the lips or in the nostrils of the patient, or the eggs may have been deposited on the food, subsequently

passing uninjured either as eggs or as young larvæ into the alimentary tract owing to insufficient mastication. Or the larvæ may have entered per rectum, the eggs having been deposited when the patient was visiting one of the old-style privies where these flies, especially *H. canicularis* and *H. scalaris*, frequently abound. These last two species are frequently the cause of this intestinal trouble, and it is most probable that the larvæ enter per rectum.

Owing to the inability on the part of the observers to distinguish the different species of dipterous larvæ we have little information as to their occurrence in these cases. Stephens (1905) records two cases. Two larvæ were procured which were stated to have been passed per rectum; one was *H. canicularis* and the other is described as *M. corvina*. The latter larva was stated to possess eight lobes on the anterior spiracular processes which "distinguishes these larvæ from *M. domestica*, which has seven only." I suspect this larva was *M. domestica*, which has six to eight lobes on the anterior spiracular processes. Some years ago a number of larvæ which had been passed by a child were sent to this laboratory, and I found that they were *M. domestica*. In 1905 some eggs taken from the stool of a patient suffering from diarrhœa were sent to me and on examination they proved to be the eggs of *C. erythrocephala*. The larvæ of the small house-fly, *H. canicularis*, as I have already mentioned, have occasionally been found in the stools of patients.

In certain cases the larvæ may wander from the mouth or alimentary tract and get into the nasal passages or other ducts, in which cases complications may ensue and result in the death of the patient.

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X. APPENDIX.

On the Breeding of *M. domestica* during the Winter Months.

In the account that I gave of the breeding habits of *M. domestica* in the second part of this monograph, it was stated (p. 503) that the experiments and observations pointed to the fact that, in the presence of suitable larval food, such as excremental matter or decaying and fermenting food materials in a moist and warm condition, the female flies would lay their eggs and the larvæ would develop if the temperature of the air was sufficiently high for the prolonged activity of the flies. Flies are sometimes found under these conditions in warm restaurants and kitchens, stables, and cowsheds, and under these conditions are able to breed during the winter months. I am pleased to find that my own observations and those of Griffith (there referred to) as to the ability of *M. domestica* to breed during the winter months has been confirmed by Jepson¹ during the past winter.

Flies were caught in February (1909) in the bakehouse of

¹ In "Reports to the Local Government Board on Public Health and Medical Subjects (New Series, No. 5). Preliminary Reports on Flies as Carriers of Infection. No. 3. Mr. Jepson's Report on the Breeding of the Common House-Fly during the Winter Months," pp. 5-8, 1909.

one of the colleges (Cambridge), and were transferred to a small experimental greenhouse in the laboratory where the temperature was from 65° F. in the morning to 75° F. in the evening. The flies were allowed to oviposit in moist bread in which the process of fermentation had begun. He found that the times for the developmental stages approximately agreed with those obtained by me at about the same temperature, and that the whole development was completed in about three weeks. At an average temperature of 70° F. the eggs were all hatched in twenty-four hours. The first larval stage lasted thirty-six hours, the second larval stage four days, and the third stage was complete in five and a half days; the whole larval period, therefore, occupied eleven days. The average period occupied in the pupal stage was ten days; some pupæ incubated at a temperature of 77° F. hatched in three days.

It may be stated now, therefore, without fear of contradiction, that flies are able to breed during the winter months, if the necessary conditions of food, temperature, and moisture are present. It is probably from these winter flies that the early summer flies are produced, as I have previously suggested.

CORRIGENDUM.

My attention has been very kindly called by Prof. W. A. Riley to a slight mistake that I have made in my account of the venation of the wing (Part I, p. 412). By an oversight I have termed transverse nervures the two small veins *m.cu.* (medio-cubital) and *cu.a.* (cubito-anal). These are really parts of the original longitudinal veins *M.* 3 and *Cu.* 2. A study of such a series of dipterous wings as those figured by Comstock in the papers there quoted (Comstock and Needham, 1898), or in his 'Manual for the Study of Entomology,' will show that these apparent transverse or cross-veins are morphologically equivalent to branches of the primary veins.

THE UNIVERSITY;
MANCHESTER.

EXPLANATION OF PLATE 22,

Illustrating Dr. C. Gordon Hewitt's paper on "The Structure, Development, and Bionomics of the House-fly, *Musca domestica*, Linn. Part III. The Bionomics, Allies, Parasites, and the Relations of *M. domestica* to Human Disease."

Fig. 1.—Mature larva of *Homalomyia canicularis*, L. $\times 17$. *a.sp.* Anterior spiracular processes. *p.sp.* Posterior spiracular apertures.

Fig. 2.—Posterior end of mature larva of *Anthomyia radicum* Mg. *an.* Anus.

Fig. 3.—Anterior spiracular process of mature larva of *A. radicum*.

Fig. 4.—Head of *Stomoxys calcitrans*, L.; left lateral aspect.

Fig. 5.—Posterior end of mature larva of *S. calcitrans*.

Fig. 6.—Posterior spiracle of the same, enlarged.

Fig. 7.—Posterior spiracle of mature larva of *Musca domestica*.

Fig. 8.—Posterior spiracles of first larval stage of *Calliphora erythrocephala*, Mg.

Fig. 9.—Posterior spiracles of second larval stage of *C. erythrocephala*.

Fig. 10.—Posterior spiracle of mature larva of *C. erythrocephala*.

Fig. 11.—Anterior spiracular process of mature larva of *C. erythrocephala*.

Fig. 12.—Posterior end of mature larva of *C. erythrocephala*.

Fig. 13.—*Chernes nodosus*, Schr. $\times 30$.

Fig. 14.—Thoraco-abdominal region of *Homalomyia canicularis*, ♀, showing Gamasids attached to the ventral side of the abdomen.

Fig. 15.—Longitudinal (sagittal) section of abdomen of *M. domestica*, which has been killed by *Empusa muscae*, showing the feltwork of fungal hyphae filling the inside of the abdominal cavity and the production of conidia in the intersegmental regions. $\times 12$. *c.* Conidiophores producing conidia. *f.* Fungal hyphae.

Fig. 16.—Four conidiophores showing the formation of conidia (*c.*). $\times 100$ (approx.).

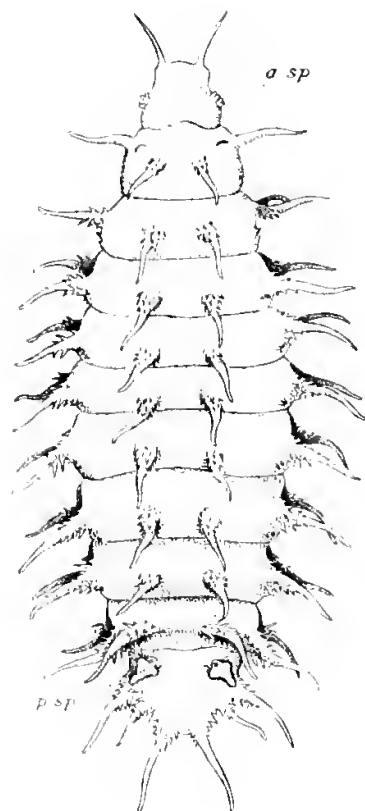
Fig. 17.—Conidium of *Empusa muscae*. $\times 400$. *o.g.* Oil globule.

Fig. 18.—*Habronema muscae* (Carter). Adult but immature specimen. $\times 85$. *g.a.* Genito-anal aperture.

Fig. 19.—Caudal end of *Habronema muscae*. $\times 360$.

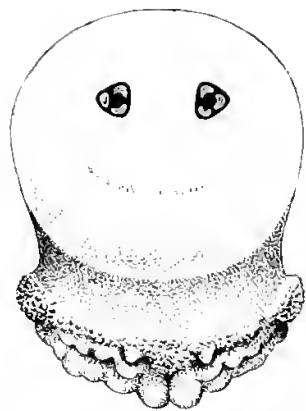
Fig. 20.—Tarsal joints of one of posterior pair of legs of *Musca domestica*. Lateral aspect, to show densely setaceous character.





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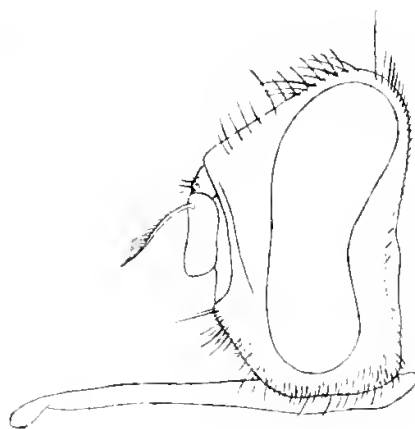
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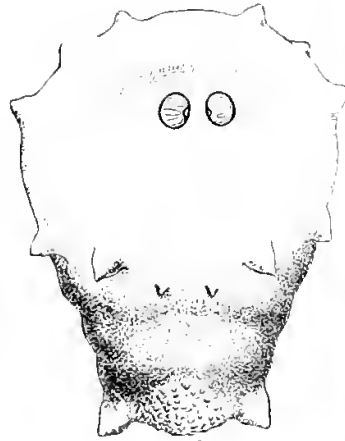
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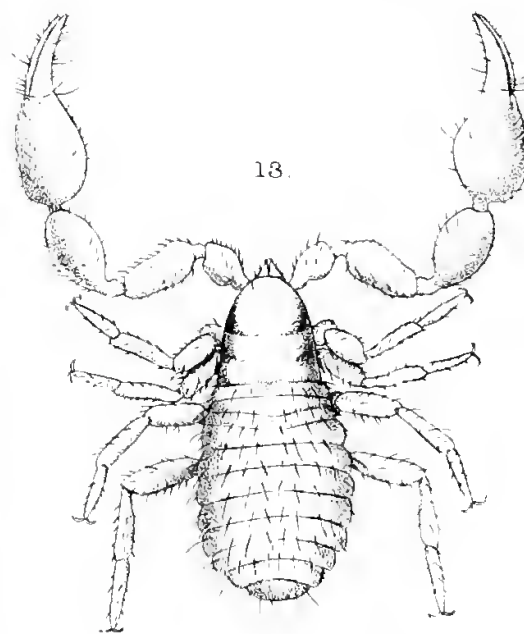
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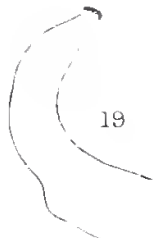
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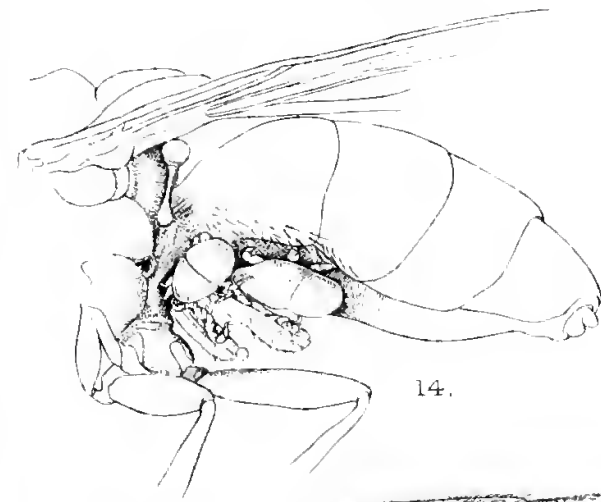
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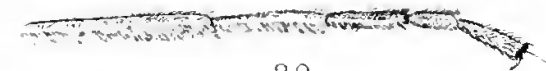


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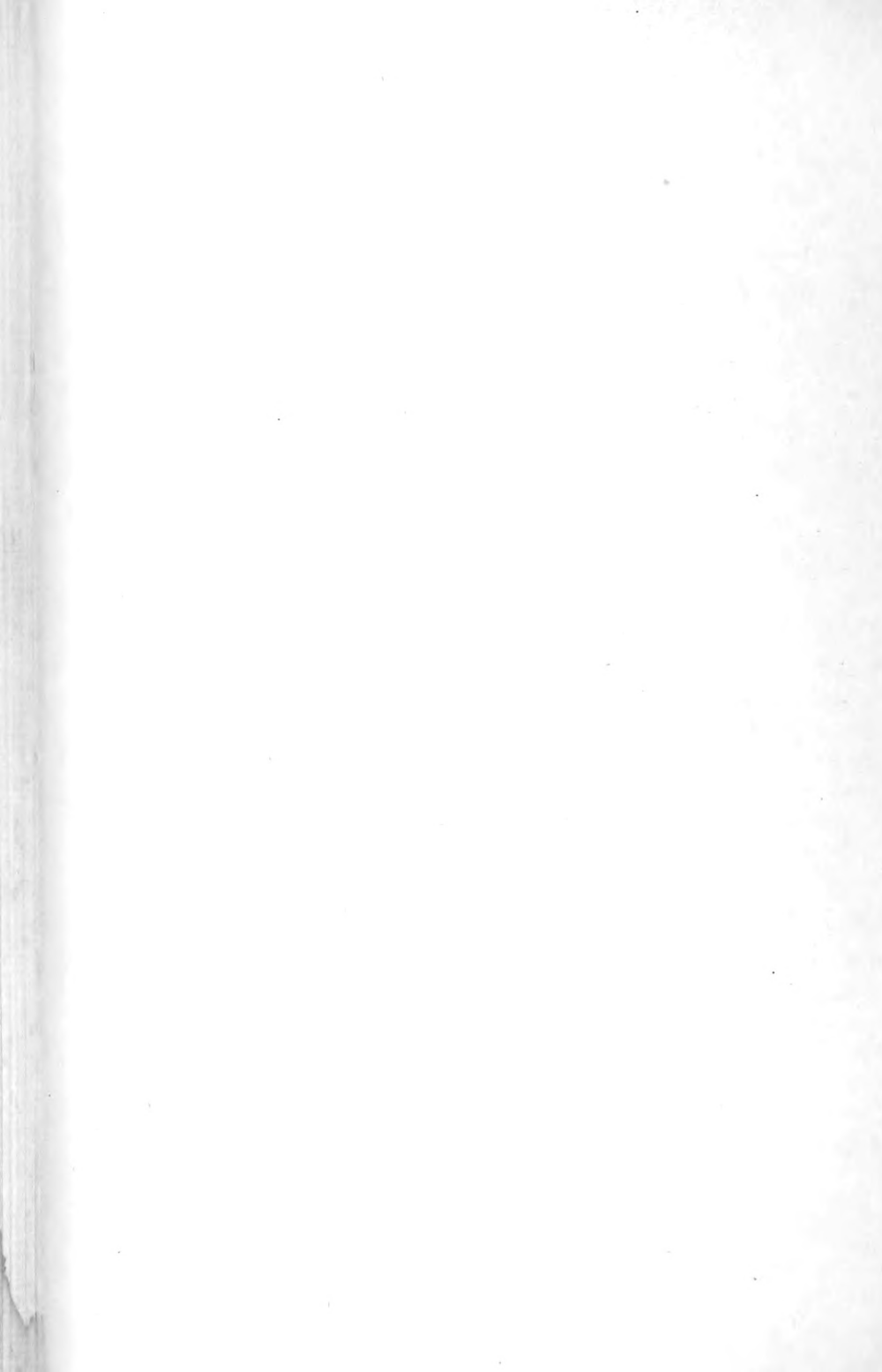


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The Development of the Temnocephaleæ.

Part I.

By

Professor W. A. Haswell, M.A., D.Sc., F.R.S.

With

Plates 23—25.

I. INTRODUCTION.

THOUGH several additions of importance¹ have been made to the literature concerned with Temnocephala and its allies since 1893, when I published an account of the group under the title "A Monograph of the Temnocephaleæ" (8), no attempt has been made hitherto to deal with the embryology of any of its members. In view of the interest which attaches to them on account of their isolated character and problematical relationships with other sections of Platodes, it is very desirable that something should be done towards filling this hiatus in our knowledge.

What follows is by no means a complete or exhaustive account of this subject. It is concerned mainly with the developmental history of a single form—viz. Temnocephala fasciata—one of the most widely distributed of the Australian representatives of the group: and in this

¹ The most extensive and important of these is that of Wacke (16), published in 1900. When writing this the author had, apparently, not had the opportunity of consulting my "Monograph," and all his references, critical and otherwise, concern themselves with a short paper which I published in 1888 in the 'Quarterly Journal of Microscopical Science.' Hence there is a good deal that goes somewhat wide of the mark.

history there are a number of important points that have not yet been determined—notably the processes of maturation and fertilisation, and the early segmentation phases. Since, however, the portions of the development which I have succeeded in tracing reveal certain phases that are quite unique in character, it appears to me expedient to publish these observations as a first contribution to our knowledge of the subject.

In addition to *Temnocephala fasciata*, *T. minor*, *T. dendyi*, and *T. quadricornis*, as well as *Craspedella spenceri*, have been made the subjects of study; but in none of these were the methods adopted with the eggs sufficiently satisfactory in their results to enable me to do more at present than state that the general course of the development is the same in all these forms. Of the somewhat specialised New Zealand species—*T. noviae-zealandiae*—I have procured ample material, which is now in course of preparation.

What has impressed me most strongly in connection with this investigation has been the complete absence in the development of any definite evidence of relationships with the groups looked upon as the most nearly allied. Until comparatively recently little that could be accepted as well authenticated had been published on the development either of the Rhabdocœles or of the Heterocotylean Trematodes. In the latter group as yet little has been done since the publication of Zeller's (17) account of the development of *Polystomum* in 1876. But Bresslau's valuable work (3) has furnished us with a much-needed body of information on certain Rhabdocœles, and this has enabled me to effect a comparison with *Temnocephala*, with the result that the differences appear to be more numerous and more radical than the resemblances. So far as can be ascertained in the present state of our knowledge, a similar result follows from a comparison with the Heterocotylean Trematodes. The upshot seems to be that the study of the development, so far as it has been carried at present, does not in any way tend to

bridge over, but rather to widen, the gap between the Temnocephaleæ and neighbouring groups.

II. METHODS.

Though the eggs are abundant and readily procurable, the study of the development of Temnocephala presents considerable technical difficulties, owing to the intractable character of the material. The egg-shell is tough and relatively thick, and not readily permeable by reagents. When it is broken through, the contents, in the fresh condition, burst out, and become completely disorganised. When fixed and hardened in the ordinary way the yolk becomes extremely hard and brittle. Many methods were experimented with before the following course of procedure, which has proved sufficiently satisfactory, was finally arrived at.

The eggs are fixed with sublimate alcohol followed by iodized alcohol and 90 per cent. alcohol. After hardening they are treated with a solution of hypochlorite of soda. If the eggs are transferred directly from the strong alcohol to the hypochlorite solution, the shell of most of them splits longitudinally, and, before the desired effect in softening and removing the egg shell has been attained, the contents, completely exposed, are disintegrated. This effect is avoided by making the transference gradually through downwardly graded alcohols to the watery solution.

A weak solution of the hypochlorite soon dissolves the cement that attaches the eggs to one another, and begins to act on the substance of the egg-shell itself. When the action is judged to have proceeded far enough, the eggs are washed in distilled water, and then dehydrated with alcohol. Double embedding is essential. From absolute alcohol the eggs are transferred to a mixture of equal parts of absolute alcohol and anhydrous ether, in which they remain for twenty-four hours. They then remain for a like period in $\frac{1}{2}$ per cent. solution of photoxylin (or celloidin) in equal parts of absolute

alcohol and ether, followed by a $2\frac{1}{2}$ per cent. solution of the same. The celloidin blocks, hardened in chloroform, are then finally embedded in the hardest paraffin in the usual way. The staining agents employed almost exclusively were Ehrlich's hæmatoxylin, or Mayer's hæmocalcium, followed by eosin.

III. FORMATION OF THE EGG.

The ovary (germarium) in *Temnocephala fasciata*, and in all the Australasian species of the genus (Pl. 23, fig. 1), is a solid ellipsoidal mass of ova enclosed in a thin capsule of muscular fibres. At the right extremity, which is the one situated nearest to the oviduct, is the largest ovum, which is more rounded in form than the rest. The remainder decrease gradually in size towards the left, the largest of them extending across the entire width of the ovary. At the left end is a mass of smaller ova, which show evidence of slow multiplication by mitotic division. The full-grown ovum, at the right-hand end of the ovary, is about 0.11 mm. in long diameter. Its protoplasm is densely loaded with very fine granules, and contains, in addition, a number of much larger rounded masses of very definite spherical form. The nucleus is large, about one third of the diameter of the ovum itself, with spherical nucleolus, and a fine, open, achromatin network.

The ripe ovum becomes detached from the others, and passes into the oviduct, which opens through the capsule of the ovary. It must then pass along the oviduct to the ootype, where it becomes surrounded by a mass of yolk-cells and the whole then becomes enclosed in a chitinous shell, the substance of which is secreted by the shell glands.

Considerable differences in detail distinguish the various species of *Temnocephala* as regards not only the male parts of the reproductive apparatus, but also the female. But in all the species which I have had the opportunity of examining, the essential features of the parts concerned in

the formation of the egg closely correspond. The two main vitelline ducts, right and left, open together into the oviduct near the ovary. Close to this the oviduct gives off a short branch leading to a large sac, with a syncytial epithelium, lying near the middle line in front of the rest of the female apparatus. This has been very usually called the receptaculum seminis—a name for which, in view of the fact that its contents consist very largely of yolk matter, I proposed (8) to substitute that of receptaculum vitelli. I was then of opinion that it serves as a receptacle in which yolk accumulates until enough has been collected for completing an egg, when it is discharged into the ootype. A more thorough examination of the subject has shown me, however, that, at least as regards the Australasian forms, both names are inappropriate. The sac usually contains spermatozoa it is true, but they never form a large proportion of its contents; and they are spermatozoa which have lost their activity, and move, when they move at all, with comparative sluggishness. It also contains yolk matter—the great bulk of the contents, in fact, consisting of that material, but it is yolk matter which has undergone degeneration; the cells have broken up, and the nuclei have, for the most part, disappeared. Moreover, mingled with the motionless or sluggish spermatozoa and the broken-down yolk-cells are shreds and strands of a substance which corresponds exactly in appearance and behaviour to staining agents with the secretion of the shell-glands. The conclusion to be arrived at from these facts is clear enough; the so-called receptaculum seminis, or receptaculum vitelli, is in reality a receptacle for surplus spermatozoa and surplus vitelline matter as well as shell-gland secretion.

The question may suggest itself—What necessity is there for such a receptacle? Why should the surplus matter not be passed directly out through the female duct? To this the answer obviously is that very frequently—whenever, in fact, an egg is in course of formation in the ootype or is lodged in the distended atrium—the way to the exterior is blocked,

and in order that the formation of the egg may proceed without interference, the yolk-cells which are being discharged into the oviduct, and the shell-gland secretion which collects after the shell has become formed, as well as the surplus spermatozoa, have to be disposed of.

That this is the function discharged by the receptaculum in *Temnocephala* there remains, to my mind, not the slightest doubt. The function of a true receptaculum seminis in those animals is discharged by the anterior part of the oviduct, in which a mass of actively moving spermatozoa is usually to be found. *T. comes* has an exceptional arrangement of the parts; in that species the cavity of the receptaculum is incompletely divided into two unequal parts by a partition which is pierced in the middle by a wide aperture. In all the specimens I have examined, while the distal larger part of the cavity, the part furthest from the oviduct, is filled with the usual mixture of effete genital products, the smaller part is occupied by a mass of normal spermatozoa.

Whether the relatively large sac known as receptaculum seminis in other *Platodes* may perform in some cases the same function as that above ascribed to it in *Temnocephala* is a question worthy of further investigation. Where, as in many Distomids, a Laurer's canal is present that canal seems to be the natural outlet for the unused and effete materials; when as in *Distomum nodulosum*, *D. globiporum*, *D. isoporum*, and others (Looss, 11), a receptaculum is present as well, it may act as a true receptaculum seminis. When a Laurer's canal is absent the receptaculum in some forms—*D. variegatum* (Looss, l.c.)—contains yolk-cells as well as spermatozoa. Looss expresses the opinion that, in general, the spermatozoa contained in the receptaculum seminis of Distomids are in process of dissolution, and no longer capable of effecting fertilisation. If this be true of the Distomids as well as *Temnocephala*, it is at least possible that the same may prove to be true of the other groups—Polyclads, Heterocotylean Trematodes.

IV. THE COMPLETED EGG.

The eggs of all the species of *Temnocephala*, on being discharged, are fixed by a chitinoid cement to some part of the outer surface of the body of their hosts. In some of the species attachment is effected through the intermediation of a longer or shorter stalk situated at one end. Such stalked eggs occur in *T. chilensis* according to Monticelli (14), Plate (15), and Wacke (16). Similar stalks occur also in *T. novæ-zealandiæ* and in *T. minor*. The cementing material usually extends between the stalks of neighbouring eggs, thus uniting them into groups as observed by Monticelli and by Wacke. In such stalked eggs an operculum may be formed, when the young animal is ready to become free, by the formation of a circular split in the egg-shell near the distal end. In both *T. novæ-zealandiæ* and *T. minor* there is a short filament attached near the middle of the distal end. *T. fasciata*, *T. comes*, *T. dendyi*, *T. semperi*, and *T. quadricornis* have more or less elliptical eggs which have no stalk, but are cemented down by one side, a number being, in most cases, united together by means of the cementing material. Of these the eggs of *T. fasciata*, *T. comes*, *T. quadricornis*, *T. semperi* are provided with filaments, those of *T. dendyi* are devoid of them.

The size of the egg is, in general, in relation with the size of the adult. The larger species—*T. fasciata*, *T. quadricornis*, and *T. novæ-zealandiæ*—have comparatively large eggs, about 5 mm. in length. The minute *Craspedella spenceri*, at the other extreme, has oval sessile eggs, without filaments, which are only 0.2 mm. in diameter.

Temnocephala, like its allies the *Rhabdocceles* and the *Heterocotylea*, has no larval stage; the young animal, when it escapes from the egg, differing from the adult only in its small size, and in the reproductive apparatus not having attained to complete development.

The older eggs are to be recognised by the eyes, which are visible through the shell. These may be placed in such a way as to show that the young *Temnocephala* is lying with its long axis parallel with that of the egg, but in a large number of cases the position is a transverse one, and occasionally an intermediate condition occurs. This variation in the direction of the long axis is not due to movement of the larva in its later stages; the direction varies from the outset, and regulating the direction of sectioning, except in advanced embryos, is little more than mere guesswork.

After the ovum has become fertilised, and the egg completed by the addition of the mass of yolk-cells and the enclosing shell, it appears to pass without much delay into the genital atrium, which serves the purpose of a uterus. Here it may remain some little time before passing out through the genital aperture; but the stage of development which has been attained when the deposition takes place varies. In most cases an egg from the ootype or atrium contains an ovum in which the process of segmentation has not yet begun; and unsegmented ova are occasionally found among those attached to the surface of the crayfish; but occasionally segmentation is found to be well advanced in a uterine egg. I have never found more than one egg in the uterus.

When the egg is fully formed, the greater part of its mass consists of yolk-cells. These are polyhedral cells of an average diameter of about $\cdot 050$ mm., with granular contents. Each has a nucleus $\cdot 015$ mm. in long diameter, of oval or elliptical shape, with a single spherical nucleolus with a diameter of about $\cdot 005$ mm. The yolk-cells are at first quite distinct from one another (Pl. 23, fig. 2); but, as development proceeds, a gradual coalescence takes place, beginning at the periphery, and eventually (Pl. 24, fig. 7) the entire mass completely fuses to form a syncytium, in which all trace of cell outlines has become completely lost. When the formation of the syncytium has begun, the nuclei of the more superficial cells pass outwards and come to lie close to the

surface, so that they present the appearance, to some extent, of the nuclei of a superficial epithelium.

The ovum (Pl. 23, fig. 2) is embedded in the yolk-cells, usually towards the middle, sometimes towards one end of the egg. It is a polyhedral cell, .08 mm. in diameter, with a finely granular protoplasm that stains much more deeply than the substance of the yolk-cells. In all the specimens I have had the opportunity of examining the nucleus has undergone modification, and is represented by a cluster of clear vesicles, each enclosing a rounded particle with the staining affinities of chromatin.¹

In two cases I found an egg containing two cells. The nuclei of both had undergone the modification just described. One of the cells was very much smaller than the other, and on that account it seems to be more probable that we have here to do with the first stage of segmentation rather than with an egg in which two ova had become enclosed.

V. EARLY DEVELOPMENT.

The process of segmentation results in the formation of a blastoderm of irregular shape, which comes to be drawn out in the direction of the long axis of the future worm—a direction, as already explained, usually parallel with the long axis of the egg, but not invariably so. No germinal layers are recognisable: but from a very early stage (Pl. 23, fig. 3) the blastoderm is found to consist of three sets of cells, which differ from one another in a very marked manner in their size and in the character of their nuclei. The cells of one set are 0.03 mm. in diameter, have nuclei about 0.015 mm. in diameter, each containing a large rounded nucleolus. Those of the second set are 0.125 mm. in diameter, have smaller nuclei, 0.0075 mm. in diameter, usually without nucleoli, but with a rather close network. The cells of the third or smallest set

¹ Somewhat similar appearances were observed by Zeller (17) in *Polystomum*.

are 0.0075 mm. in diameter, with correspondingly small nuclei. No definite arrangement of these cells is recognizable until a few of the larger cells become grouped together (Pl. 24, fig. 5) in such a way as to bound a small rounded cavity. This elongates and widens (Pl. 24, fig. 6), the bounding cells meantime increasing in number. Eventually (Pl. 24, figs. 7, 8, 9, and 10) the space dilates very greatly, the cells which form its walls becoming correspondingly extended, and uniting together to form a comparatively thin membrane with flattened nuclei. As it enlarges, this space becomes approximated towards the surface of the egg, coming to be separated from the shell only by a thin layer of yolk. In apposition with the deeper side of the space lies the main mass of the blastoderm, which is rapidly increasing in extent, the increase being mainly due to the multiplication of the middle-sized cells.

The space above referred to does not correspond, so far as I have been able to ascertain, to anything that has been found to occur in any other group of animals. Since it plays an important part in development, it is necessary to have a name for it, and I propose the term *endocœle* as one not involving any dubious homologies.¹

The rudiment of the brain (Pl. 24, figs. 8, 9, and 10, *br.*) makes its appearance as the *endocœle* approaches its maximum size. It appears first as a bilobed, dense aggregation of cells on the deeper dorsal side of the *endocœle*, a little distance from the lining membrane. In the middle of this appears a transversely elongated space filled with finely fibrillated matter—"Punktsubstanz." Nerve-fibres (or nerve-tubes) are only developed in the latest embryonal stages. From the central mass a pair of processes—the foundations of the peripheral nervous system—are given off laterally.

About the same time as the beginnings of the nervous system, appears the first rudiment of the excretory system of vessels. This takes the form of several specially modified

¹ The same cavity with the same relations occurs in *Craspedella* as well as *Temnocephala*.

large cells, on either side of the rudiment of the brain, and somewhat behind it. These cells are situated immediately below the thin epithelial lining of the endocœle cavity. One cell becomes considerably enlarged, and a narrow sinuous channel becomes formed in its substance. This channel becomes continued through a second and a third cell placed in close apposition with the first. As subsequent stages show, these constitute the rudiments of the terminal contractile sacs and the beginnings of the main vessels of the excretory system.

The pharynx is formed from a number of cells which become arranged after the manner of an epithelium immediately beneath (i. e. outside of) the thin syncytial epithelium of the endocœle on the dorsal side. The time of appearance of this layer varies somewhat. Usually it is not seen until both the brain and excretory rudiments have become well established. The part of the wall of the cavity on which it is situated becomes somewhat rounded off, though still remaining in wide communication with the rest. Posteriorly a short prolongation without cellular lining extends for a short distance backwards into the mass of yolk; this represents the lumen of the intestine (see Pl. 24, fig. 11).

The cavity of the endocœle as a whole decreases much in size. The thin layer of yolk by which it is separated from the exterior becomes still more attenuated, but still remains as a definite septum (Pls. 24 and 25, figs. 11, 12, 13, s.), cutting off the whole internal cavity from the exterior.

When the brain and the excretory sacs are first formed, the blastoderm does not extend anteriorly or posteriorly beyond the limits of the endocœle. But a little later it begins to grow backwards to form the foundations of the posterior parts of the embryo. This backward extension (Pl. 25, fig. 14) is made up, like the main body of the blastoderm, of cells of three sizes, small, intermediate, and large; and these are arranged in groups with a marked bilateral symmetry. The large cells are formed by a proliferation of the membrane lining the endocœle. Outlying small cells are to be found

here and there embedded in the yolk, at a little distance from the main body.

In the next stage observed this posterior elongation of the blastoderm has reached the ventral surface, in what is destined to be the genital region, and has become almost completely separated off from the anterior part, in which the further development of the brain, the pharynx, and the excretory system is going on. There thus come to be two distinct foci of development, an anterior and a posterior. From both superficial cells must be separated off to form the epidermis, since this layer is to be recognised as a distinct, though very thin layer, with widely separated nuclei, at the stages when the tentacles and sucker first begin to be formed. This layer is early completed on the ventral surface, but on the dorsal surface it does not make its appearance till a considerably later stage.

The rudiments of the tentacles (Pl. 24, fig. 11) make their appearance at a stage when certain changes in the cavity have led to the first differentiation of the pharynx, and when the rudiments of the eyes have first become distinguishable. They appear as processes which grow at first straight forwards from the anterior extremity of the body; but as they elongate, they become bent (Pl. 25, figs. 12, 13), usually towards the ventral, but in many cases towards the dorsal, side. In the former case the cephalic portion of the body (Pl. 25, fig. 13) becomes strongly flexed ventrally at the same time. When this takes place the part of the surface covered by the reflexed tentacles develops a system of minute tooth-like epidermal papillæ, which are apparently of firm consistency.

The rudiment of the sucker makes its appearance about the same time as those of the tentacles. Epidermal papillæ, similar to those underneath the tentacles, are formed under the sucker.

We have left the endocœle as an extensive rounded space, occupying nearly a third of the length of the egg, with a floor and a roof. As the cavity reaches its greatest dimen-

sions the layer of yolk, which separates it on the ventral side from the exterior, becomes greatly reduced in thickness. At the same time, subsequently to the formation of the embryonic brain and excretory sacs, a portion of its wall just behind the brain undergoes modification, a number of large cells becoming arranged in an epithelium-like manner beneath the thin lining membrane. The cavity then becomes much reduced in size, and the part with the large cells becomes rounded off to form the pharynx. Ventrally it continues to be separated from the exterior by the original roof of the cavity, which becomes reduced to an extremely thin membrane. Posteriorly a further remnant of the original cavity is represented by a very short passage which ends blindly in the yolk. The pharyngeal sac and mouth become formed by growth of the integumentary and muscular layers around the thin membrane that represents the original roof of the cavity; but the membrane remains as a distinct, though very delicate, partition between the buccal cavity and the pharynx as long as the young animal remains within the egg.

The intestine remains without lumen in the most advanced stage observed within the egg, a stage in which all the other parts, even the male reproductive apparatus, have reached an advanced stage of development. It would, perhaps, be more correct to say that the young animal has no intestine at this stage, the site of the future intestine being occupied by a solid mass of yolk still containing remains of the original nuclei of the yolk-cells.

VI. DEVELOPMENT OF THE EXCRETORY SYSTEM.

The excretory system of the adult *Temnocephala*, though constructed on the same type as that of the *Platodes* in general, possesses certain features which, so far as our present knowledge extends, are peculiarly its own. The presence of contractile terminal sacs through which the system communicates with the exterior is not peculiar to

this group, similar structures occurring in some of the Heterocotylean Trematodes (Braun, 2; Goto, 4). But the nature of these sacs and their relations to the system of vessels in *Temnocephala* are quite unlike anything that is known to occur in other forms.

The most essential features of this system were described by me in 1893 (8). But since some of the most important of these points have been overlooked by recent writers,¹ or their significance not recognised, it seems desirable to give a brief résumé of the facts.

Each of the two dorsally situated excretory apertures leads into a thick-walled contractile terminal vesicle (Pl. 25, fig. 18), which is of pyriform shape, bent on itself towards its apex. The contractions of the walls of the vesicle are effected by the agency of an enclosing layer of muscular fibres, and the external aperture is surrounded by a muscular sphincter. Between the muscular layer and the proper wall of the vesicle is a layer of loose parenchyma, which doubtless facilitates freedom of movement. The vesicle itself consists of two large cells fused together and hollowed out to form the lumen. The greater part of the wall of the vesicle is formed by one of these two cells; the narrower apical part, and the beginning of the main duct which is given off from it by the other. The relative position of the two cells is indicated mainly by the position of their two nuclei, but the texture of the protoplasmic substance of the two cells differs somewhat in character, and that of the smaller is much more susceptible to the action of staining agents.

The inner surface of the vesicle is quite smooth and uniform. Where an occasional exception to this appears to occur, and the usually sharp internal outline appears blurred,

¹ Plate (15) and Wacke (16) for example. The former states, "An den Nephridien der *Temnocephaliden* ist bis jetzt vergeblich nach den für die *Platyhelminthen* so charakteristischen Flimmerzellen gesucht worden." Yet I had described the system with its flame-cells in a paper in the 'Zoologischen Anzeiger' two years before (1892).

and the lumen is occupied by fine filamentous matter that might be mistaken for cilia, this occurs in such an irregular way that I have little doubt that this appearance is due to a rupture or other alteration that has occurred during the fixing process. In no case, either in *Temnocephala novæ-zealandiæ* or any other form, is there an epithelium with scattered nuclei, as supposed by Wacke. In some preparations the internal contour appears double, as if the cavity of the vesicle possessed an excessively thin cuticular lining, but this is always very indefinite, and in many cases is not to be detected. The only nuclei in the entire organ are the two already referred to as the nuclei of the two constituent cells.

Arising from the main excretory trunk at a little distance from the vesicle is a special branch of considerable size—the vesicular vessel as it may conveniently be termed. This runs inwards, and enters the wall of the vesicle on its inner side. Here it breaks up (Pl. 25, fig. 19) into a number of branches, which ramify throughout the protoplasmic substance of the wall of the sac in all directions. In the course of the system of fine intracellular capillaries which is thus formed occur numerous ciliary flames of small size, but in other respects similar to the ciliary flames in the flame-cells of other *Platodes*. I have counted as many as fifty of these ciliary flames in movement at one time in the case of *T. novæ-zealandiæ*, and probably many more than that number are actually present.

In sections of the terminal vesicle in all the Australasian species the ramifications of the vesicular vessel are very conspicuous, pervading the protoplasmic wall in all directions. But the ciliary flames are not to be made out with any certainty save in the living animal.

In most cases the inner part of the protoplasmic wall in sections appears regularly divided by fine parallel vertical lines, and one might be tempted to suppose that these represent a system of vertical canals forming outlets from the system of vesicular capillaries into the lumen of the vesicle.

From the unbroken appearance of the limiting line of the surface on which the vertical lines terminate, I incline to the opinion that no such communications exist.

The details of the arrangement of the vessels differ in the different species. The main trunk soon bifurcates to form anterior and posterior main vessels, which give off numerous branches to all parts of the body. A large vessel runs along the axis of each of the tentacles.

Given off from the larger vessels in the body is a system of fine, thin-walled capillaries, which are most abundant near the dorsal surface, where they form an extensive plexus.

A limited number of ciliary flames are to be detected in the living animal distributed throughout various parts of the body and the tentacles. The relation of these to the vessels of the excretory system still remains undetermined. In no case was a nucleus observed in close relation with the ciliary flame.

The walls of the larger vessels consist simply of a fine-grained, structureless protoplasmic material. Here and there, usually at long intervals, are the nuclei of the elongated cells of which the walls are composed. These are comparatively few in number even in the larger species. Their presence and their relations to the vessels are best observed in longitudinal sections—most readily in the tentacles, in which such appearances as those represented in figs. 16, 17, 18 of pl. x of my "Monograph" are readily recognisable.

Some of the excretory vessels end in certain specially modified large excretory cells. The branch in question, sometimes fairly thick-walled, sometimes very delicate, enters the cell and breaks up into a richly ramifying and anastomosing system of minute capillaries within its substance.

The first trace of the system of excretory vessels makes its appearance at a very early stage in the history of the embryo. In a blastoderm in which the endocoele has become developed, but is still very small, and is bounded by thick massive cells, there may be observed (Pl. 25, figs. 15 and 16) on each side in close apposition two cells which have the appearance in

section of being pierced by an exceedingly fine, perfectly clean-cut canal. How these canals end I have not been able to determine: but subsequent stages show that these, with the cells that contain them, are the foundations of the excretory system.

At first these perforated cells are widely separated from the endocœle cavity and embedded in the thickness of the blastoderm. As the cavity increases in size their relative position becomes altered, until they come to lie directly below the lining membrane of the cavity. At first the entire structure consists on each side of two cells, a larger and a smaller, which have fused, and the substance of which has become perforated by a sinuous canal. This is destined to give rise to the terminal sac of the excretory system. The canal extends through several cells situated close to the first two, and in this way is formed the beginnings of the main longitudinal vessels. Later (Pl. 25, fig. 17), when the rudiment of the brain has become well advanced, the terminal sacs, while still retaining their position immediately under—i. e. external to—the membrane lining the cavity, and, while still continuing each to consist of only two fused cells perforated by a canal, assume a more complex structure, and take on, in all the most essential points, the structure which we have found to characterise them in the adult. The canal becomes wider in relation to the thickness of the enclosing wall; and from the main vessel is given off a slender branch—the future vesicular vessel—which, approaching the terminal sac on the inner side, breaks up into a number of excessively fine capillaries that ramify through the substance of the wall of the sac.

It will be seen from the above account of its mode of formation that the excretory system of *Temnocephala* is, in the strictest sense, of intracellular character. I am thus compelled to dissent from Goto's opinion (4, p. 71) that I was not justified in using that term, as well as to his more general view (p. 74) that "the term intracellular is quite in-

appropriate to the excretory system of the Trematodes and the Turbellaria."

Perhaps the most remarkable event in the history of the development of the excretory system in *Tennocephala* is the change which takes place in the position of the contractile terminal sacs. Originally, as we have seen, they are placed close to the epithelium of the endocœle, and apparently open into the latter. When the cavity becomes reduced, and the pharynx begins to become rounded off, the sacs lose their original connections, and, becoming displaced outwards, enter into connection with the epidermis, and open on the exterior on the dorsal surface. Thus, in a stage in which the rudiments of the tentacles are being formed, the sacs occupy the position which they retain in the adult.

VII. DEVELOPMENT OF THE ALIMENTARY SYSTEM.

The alimentary system of *Tennocephala* consists of two principal parts—pharynx and intestine. The mouth, situated far forwards on the ventral surface, leads, through a very small cavity representing a pharyngeal sac, into the lumen of the pharynx. The latter is a large, rounded organ with thick walls of highly complex structure. The cavity is lined internally by a layer, the nature of which is by no means clear. By Weber (18) it has been described as a continuation of the cuticle of the integument. Monticelli (14) refers to it as a syncytial epithelium. Wacke (16) refers to it as an epithelium, and gives excellent figures of its minute structure. It is a non-cellular layer, in which a degenerate nucleus may sometimes be detected, but only quite exceptionally. It is composed of granular material, the granules of which are arranged in rows or strings, most, at least in the Australasian species, having a vertical direction. Many of these strings are traceable in some series into the thickness of the wall of the pharynx. The internal cuticle, described and figured by both Monticelli and Wacke as

bounding this layer internally, is not a separate and distinct layer, but is merely the innermost stratum of the granular layer, and in most preparations is not differentiated at all.

Externally a limiting membrane forms a pharyngeal capsule, separating the muscular mass of the pharynx from the surrounding parenchyma. Between the external capsule and the internal epithelium, in addition to the elaborate system of muscular fibres, there are a number of cells and a system of nerve-fibres. The cells are of several kinds, comprising bipolar nerve-cells, excretory cells, and unicellular glands, the ducts of which are usually said to open into the cavity of the pharynx.

Posteriorly the pharynx leads through a short passage—the œsophagus—into the spacious intestine. Round the œsophagus are a number of unicellular “salivary” glands.

The intestine in all the Australasian species (and also in *T. chilensis* according to Wacke) is constricted at intervals by a number of annular muscular dissepiments, the number of these varying with the species. The epithelium is composed of long narrow cells, the majority of which, though probably mainly absorptive in function, contain vacuoles enclosing granules which are probably excretional, while others are of the nature of unicellular digestive glands.

The pharyngeal sac, the pharynx with the œsophagus, and the intestine are all derived from different sources. The first may be said to be of the nature of a stomodæum. The pharynx is derived from a portion of the wall of the endo-cœle. A number of large cells become arranged in a manner presenting the appearance of an epithelium on the wall of this region, separated from the internal space by the thin epithelial lining of the cavity. This specially modified portion of the wall then becomes rounded off as the wall of the pharynx, what remains of the cavity, which becomes much diminished in size, forming a short passage corresponding in position with the future œsophagus, and terminating abruptly behind in the mass of yolk.

The large cells in the wall of the embryonic pharynx at

first form its entire thickness, with the exception of the exceedingly thin internal epithelium. But afterwards muscular fibres are developed both internally and externally. The mode of formation of these is not clear, but since no other elements come into play, there can be little doubt that a portion of the cells of the wall of the pharynx are of the nature of myoblasts. The rest become the nerve-elements and the glandular and excretory cells.

The mode of formation of the so-called epithelium of the pharynx is a matter of some interest. It is represented at first, as already pointed out, by the thin syncytial lining of that part of the endocœle from which the pharynx becomes developed; and at no subsequent stage does its epithelial character become more pronounced. On the contrary, as development advances, the cellular character of this layer becomes almost or completely lost. In fact, were it not for the occasional occurrence in it of a more or less altered nucleus, it might be supposed to be entirely non-cellular. But, though it loses, or nearly loses, its cellular character, this layer greatly increases in thickness, and, in the adult, maintains its thickness in spite of the loss of material to which it must be subjected as a result of constant wear and tear during the capture and ingestion of active living prey.

Up to a late stage in *Temnocephala fasciata* the rest of the digestive system is merely represented by a short passage, which continues back the lumen of the pharynx, and ends abruptly within the mass of yolk. This is the future œsophagus, and in its neighbourhood are a number of cells destined to become the unicellular or "salivary" glands. Thus, at a stage when the muscular wall of the pharynx, with its anterior and posterior sphincters, has reached an advanced stage of development, the intestine is not yet definitely represented.

At this stage the cavity of the pharynx is still completely shut off from the exterior by the septum formed, as already described, from the persistent thin roof of the original endocœlc.

The stages by which the intestine, with its definite epithelium and septa, becomes differentiated, have not yet been traced. It seems probable, however, that a structure which makes its appearance below the endocœle, after the brain and excretory sacs have become formed, may be concerned in the formation of the primitive endoderm. At this stage (Pl. 25, fig. 20), a cleft or infolding appears among the cells on the floor of the cavity. Later this takes the form of a group of cells arranged around a small lumen—the appearance being very similar to that presented by the first beginnings of the endocœle itself. Since this is behind the brain and excretory sacs, and is too far forward to be of the nature of a genital primordium, it is permissible to suppose that it represents the earliest rudiment of the endodermal system. Its further history has not been traced; in fact, it soon disappears as such; but if the above supposition as to its nature be correct the cells to which it gives rise must extend round the yolk between it and the rest of the developing parts, and become converted into the intestinal epithelium.

In the latest stage observed within the egg—a stage with fully-developed eyes and abundant pigment, and with the male part of the reproductive apparatus far advanced—the intestine is still entirely devoid of lumen, and consists of a solid granular mass with numerous nuclei, which are most abundant in the peripheral zone. Slender strands passing inwards into this solid mass represent the future dissepiments. In the case of *T. novæ-zealandiæ*, however, in the latest stages from the egg the intestine has developed a lumen, and the epithelium is recognisable, though the septum still persists shutting off the cavity of the pharynx from the exterior. It is a remarkable fact, which is probably of significance in connection with the function of this persistent septum, that in nearly all sections of late stages with fully-developed pharynx the lumen of the latter is found to be filled with a mass of yolk-granules which have evidently become detached from the central body of yolk, and have been prevented by

the occluding septum from reaching the exterior, i. e. the space between the embryo and the shell.

VIII. DEVELOPMENT OF THE EPIDERMIS.

In the integumentary system of *Temnocephala* the most characteristic feature is the presence of a well-developed nucleated epidermal layer of a syncytial character. In the embryo this is not represented by any definite primordium, such as has been observed to be formed in *Polyclads* (Lang and others) and *Rhabdocœles* (Caullery and Mesnil [4], Breslau [3]), and there is no process corresponding to the process of overgrowing of the embryo by an epidermal layer, such as occurs in these groups. When an epidermis is first discernible, it consists of a very thin membrane with wide-apart flattened nuclei, covering only the ventral surface, and there is no evidence of any process of proliferation such as must accompany the spreading of the edge of this layer by cell-division. It would appear, in fact, as if the epidermis were formed by cells migrating to the surface and there becoming flattened out and united together to form the syncytium.

Temnocephala fasciata, unlike *T. minor* and *T. Dendyi*, has no cilia on the surface in the adult, and I have found no trace of them at any stage in the development.

IX. DEVELOPMENT OF THE REPRODUCTIVE SYSTEM.

The details of this process have not yet been followed out. In all the species examined the male part of the reproductive apparatus is developed at an earlier stage than the female. In the later stages within the egg the penis, the vesicula seminalis, the vasa deferentia, and the testes are all well advanced. In *T. fasciata* each testis is represented by a

mass of primordial cells surrounded by indifferent cells. In *T. novæ-zealandiæ* before the young animal leaves the egg the first stages of spermatogenesis have occurred. At this stage the atrium does not yet open on the exterior. The definite development of the female part of the apparatus does not begin till after hatching, so that Wacke's statement that *Temnocephala* is protandrous appears to have some evidence in its favour, though a study of the post-larval development will be necessary in order to decide whether the condition is one of actual functional protandry, and not merely one of more active development of the male apparatus in the early stages.

The unique features of the early development of *Temnocephala* are associated with the formation of the remarkable internal cavity (endocœle) around which the foundations of various systems of organs are laid down. As nothing parallel to this cavity has, so far as my knowledge extends, been met with in other groups,¹ it is natural to inquire if its presence can be associated with any special conditions under which the development takes place—if its occurrence can be supposed to be of the nature of an adaptation.

The Australian fresh-water Crayfishes, on which live all the known Australian members of the *Temnocephalæ*, shelter or support a great variety of small Invertebrata. A considerable proportion of these live in the branchial cavities, but many adhere to various parts of the outer surface. Among such dependents of the Crayfishes are Protozoans, Nematodes, Rhabdocœles, Rotifers, *Stratiodrillus*, *Phreodrillus*, a parasitic Hydrachnid, and others. In a country subject at times, as Australia is in many parts, to long-continued droughts,

¹ It might be possible to trace some connection between the embryonal pharynx of the *Tricladida* (Hallez [6], Metschnikoff [12], Iijima [10]) and the endocœle of *Temnocephala*. Both cavities arise as spaces in rounded groups of cells in the blastoderm; but the former opens on the surface and swallows yolk cells, with which the intestine becomes distended. Moreover it is purely provisional, and disappears entirely, a new pharynx becoming developed in its place.

aquatic organisms must often suffer wholesale destruction as a result of the drying-up of the smaller streams. Crayfishes are able to avoid such a fate by sheltering between boulders or burrowing deeply in the bed of the stream. The animals adhering to them thus have a chance of survival denied to their free-living relatives. Yet it may, and doubtless does, often happen that even the Crayfishes are unable to escape the risk of desiccation. Under such circumstances the presence of a relatively large space filled with water in the interior of the egg of the *Temnocephaleæ* may make all the difference in enabling the embryo to retain its vitality until the dry period passes and the stream fills again.

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¹ I have not been able to see this paper. What I have learnt regarding its contents has been obtained from Graff (5).

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EXPLANATION OF PLATES 23—25,¹

Illustrating Professor W. A. Haswell's paper on “The Development of the Temnocephaleæ.”

LETTERING.

br. Brain. *e.* Supposed endoderm primordium. *en.* Endocœle. *ep.* Epidermis. *ep. en.* Epithelial lining of endocœle. *ex.* Excretory sac or excretory cells. *ey.* Eye. *o.* Ovum. *o. p.* Oral papilla. *ph.* Pharynx. *s.* Roof of endocœle, becoming septum, cutting off lumen of pharynx from the exterior. *t.* Tentacle. *y.* Yolk-cells. *y'.* Yolk syncytium.

PLATE 23.

FIG. 1.—Longitudinal section of the ovary of *Temnocephala comes*. × 500.

¹ All the figures have been re-drawn by Mr. A. C. Cronin, of the Water and Sewerage Department, Sydney, from my original drawings.

FIG. 2.—Transverse section through uterine ovum of *T. fasciata*, passing through the unsegmented ovum. $\times 250$.

FIG. 3.—Section through an early blastoderm in which three kinds of cells have become distinguishable. $\times 500$.

PLATE 24.

FIG. 4.—Section through a later stage, with groups of larger, lighter, surrounded by smaller, more darkly stained, cells. $\times 500$.

FIG. 5.—Section through a blastoderm in which the first definite trace of the cavity has made its appearance. $\times 500$.

FIG. 6.—Later stage in the development of the cavity. $\times 500$.

FIG. 7. Longitudinal section through the entire egg, with a more advanced cavity. $\times 170$.

FIG. 8.—Portion of a longitudinal section of egg passing through the cavity, and cutting the blastoderm nearly transversely. \times about 330.

FIG. 9.—Similar section of a later stage with the brain more advanced. \times about 330.

FIG. 10.—Similar section, cutting the blastoderm nearly transversely, but with some obliquity, so that it passes through the brain and one excretory sac. $\times 330$.

FIG. 11.—Anterior part of a vertical longitudinal section, approximately median, at a stage when the rudiments of the tentacles and eyes are appearing. \times about 330.

PLATE 25.

FIG. 12.—Entire longitudinal and vertical section of embryo with the tentacles further developed than at the stage represented in fig. 11, and flexed backwards over the mouth. \times about 130.

FIG. 13.—Anterior part of a longitudinal and approximately median and vertical section of an embryo with well-developed, ventrally flexed tentacles.]

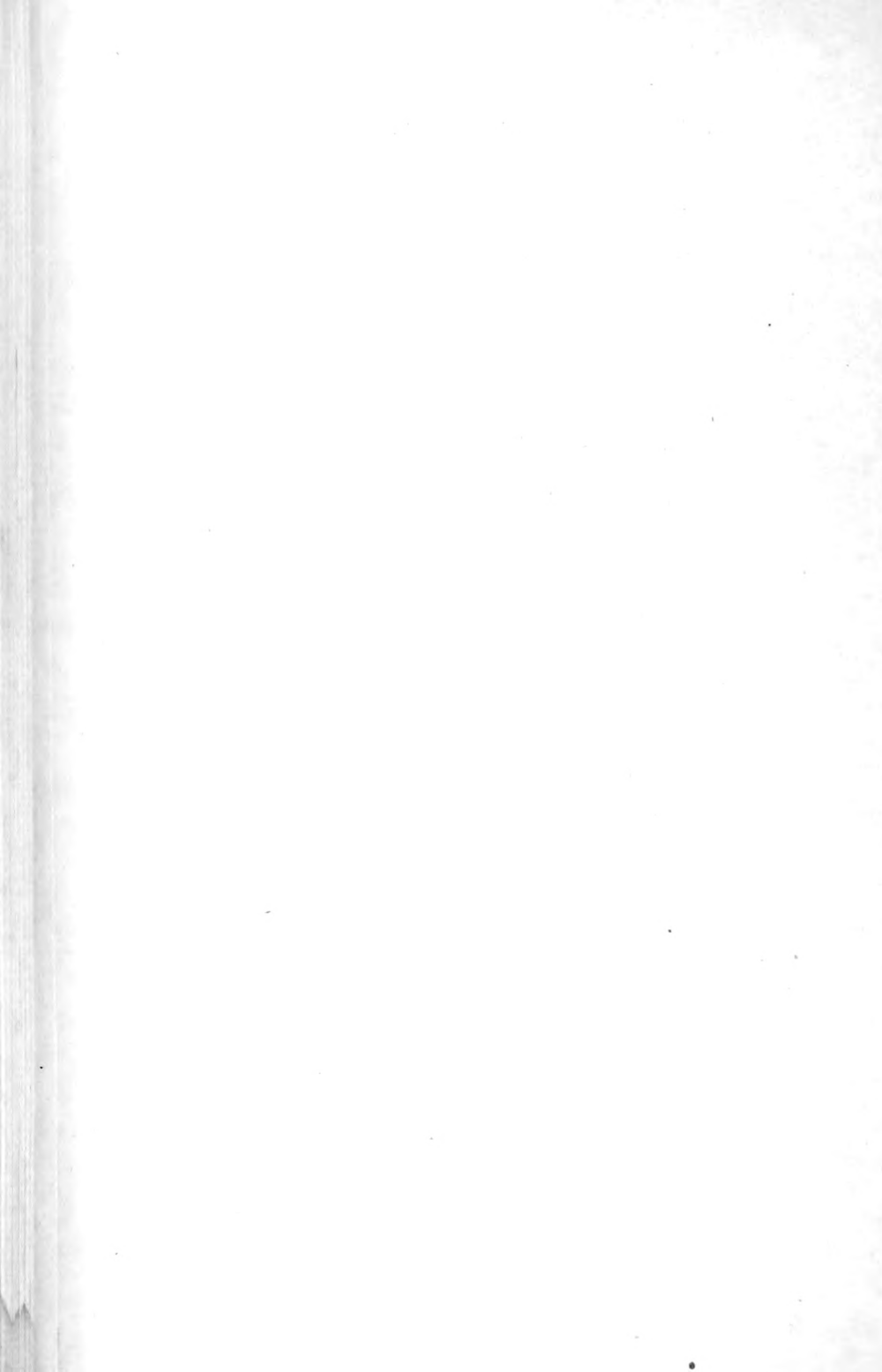
FIG. 14.—Transverse section through the blastoderm in the region behind the endocœle at a stage somewhat later than that represented in fig. 10, showing the posterior prolongation comprising the genital rudiment. \times about 500.

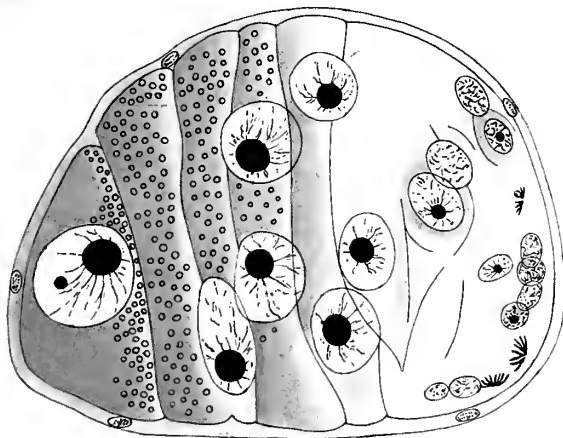
FIGS. 15 and 16.—Successive sections of an embryo at an early stage in the formation of the endocœle, showing an early phase in the history of the cells destined to form one of the excretory sacs. \times about 500.

FIG. 17.—Portion of a section passing through the endocœle at a stage similar to that represented in fig. 9, showing the developing excretory sac, and its connection with the endocœle.

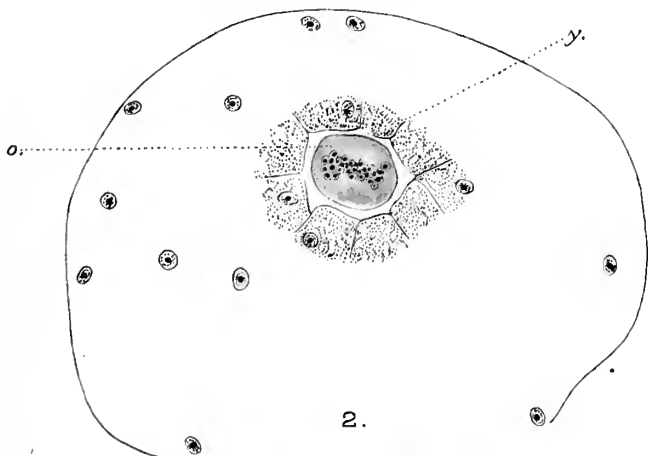
FIGS. 18 and 19.—The excretory sac in the adult: fig. 19 shows a part of the system of capillaries in which are numerous flame-cells, given off from the vesicular vessel and ramifying through the wall of the sac. Reproduced from a 'Monograph of the Temnocephalæ,' pl. x, figs. 11 and 12.

FIG. 20.—Portion of a section of an egg with far advanced endocœle and distinct brain and excretory sacs, showing what is supposed to be the first trace of an endodermal primordium.

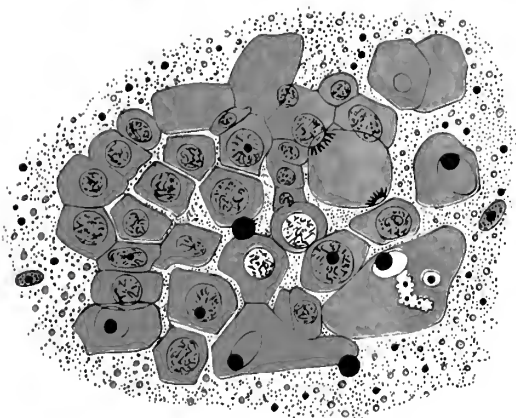




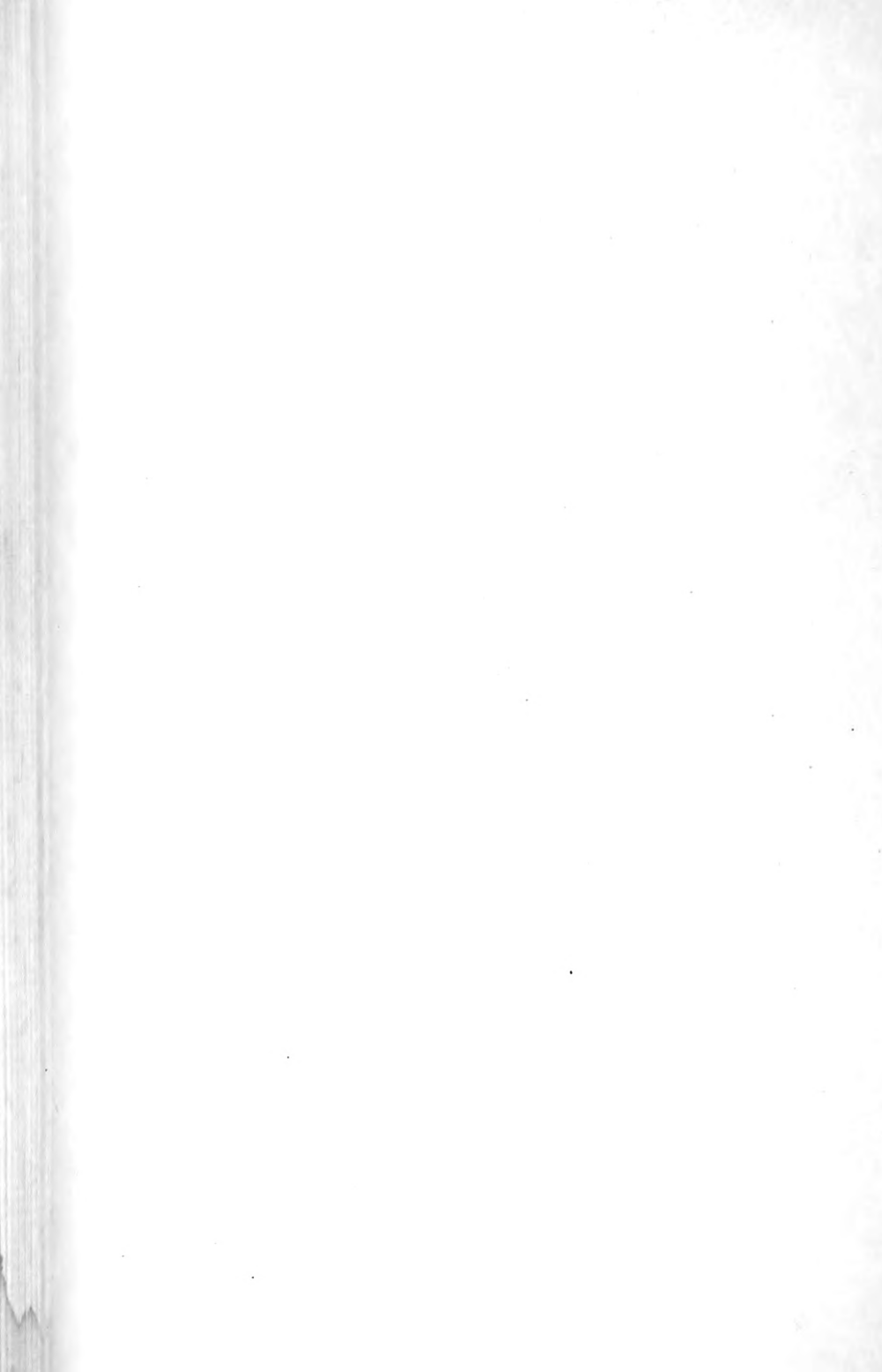
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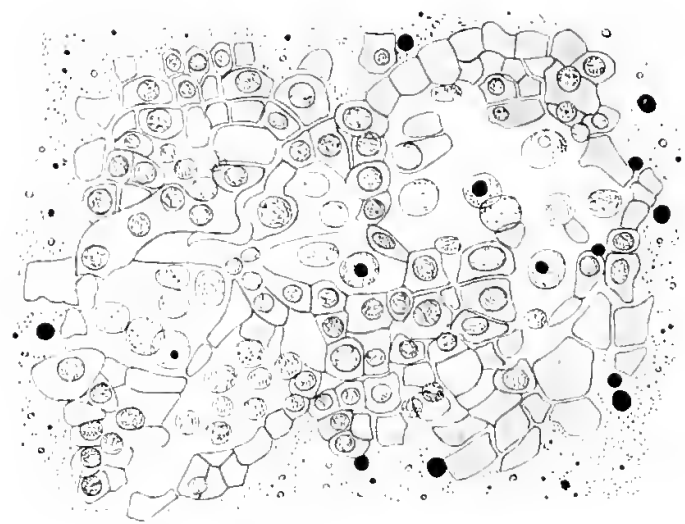


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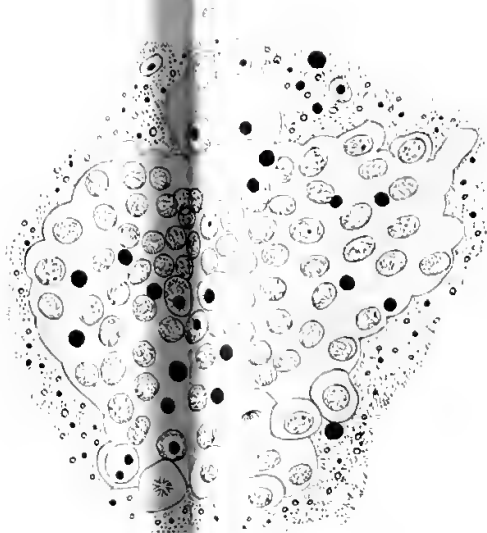
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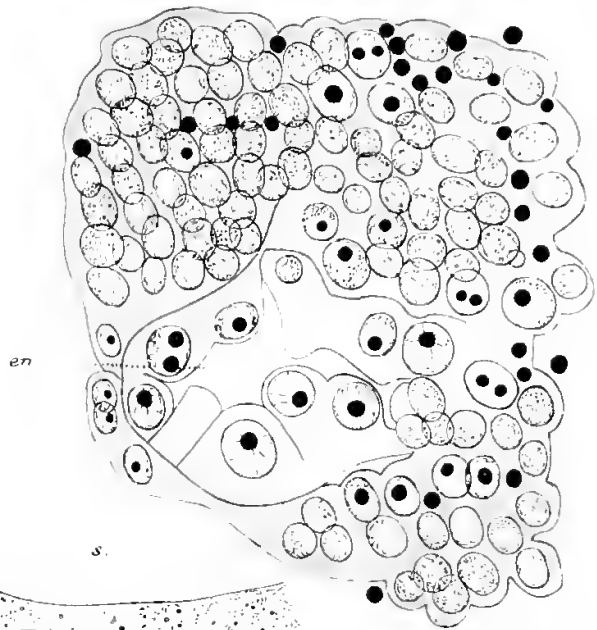


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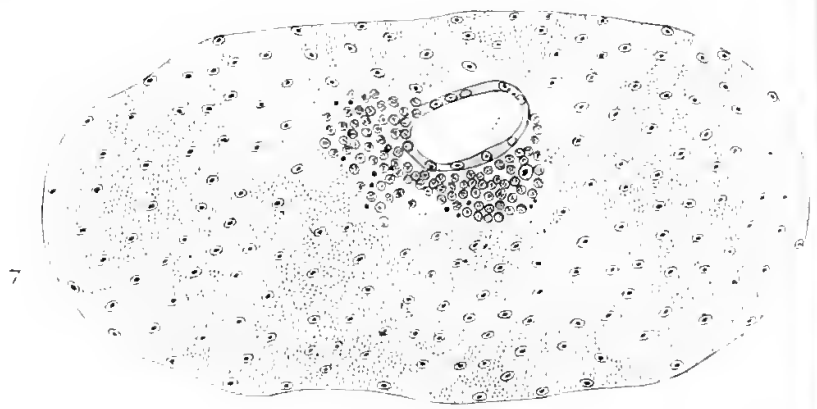
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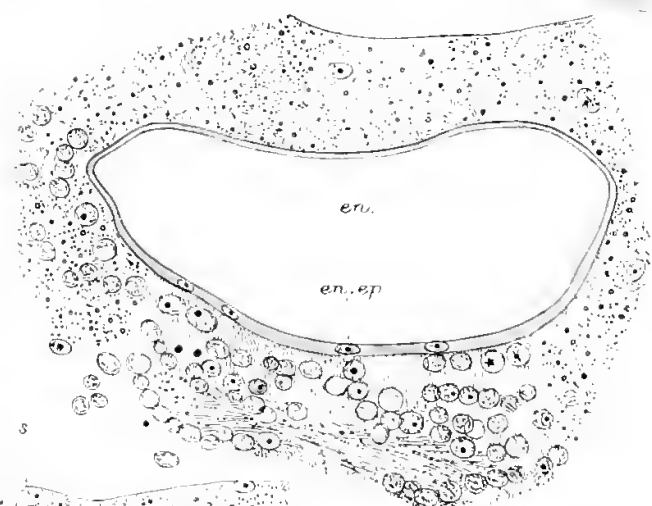
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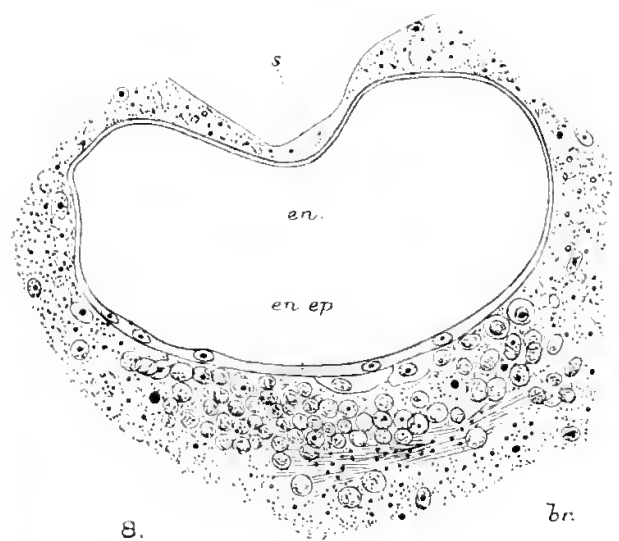


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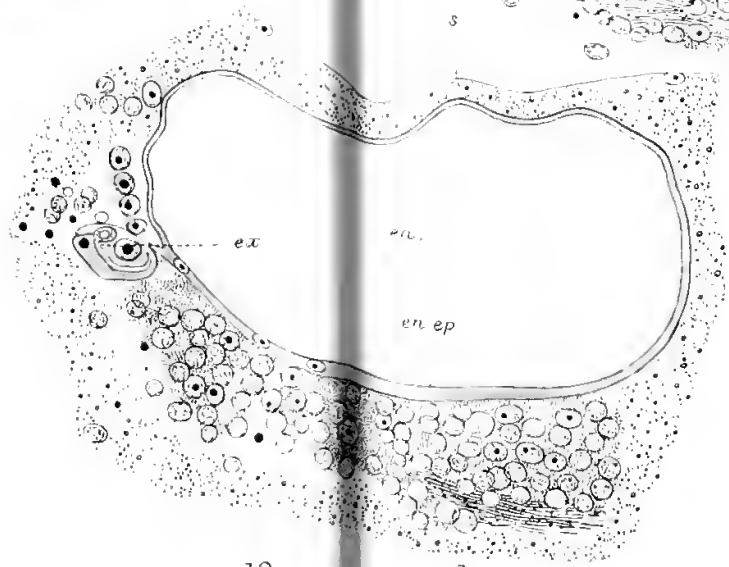
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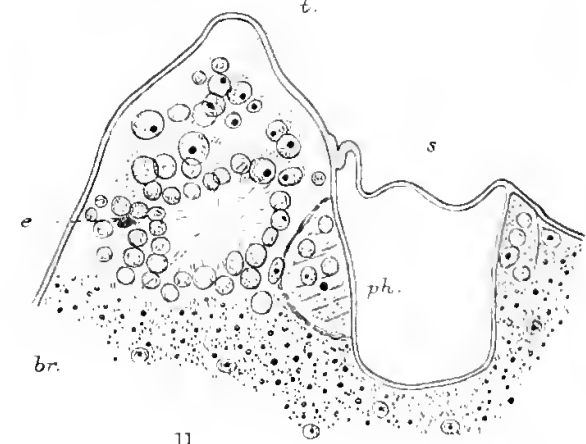
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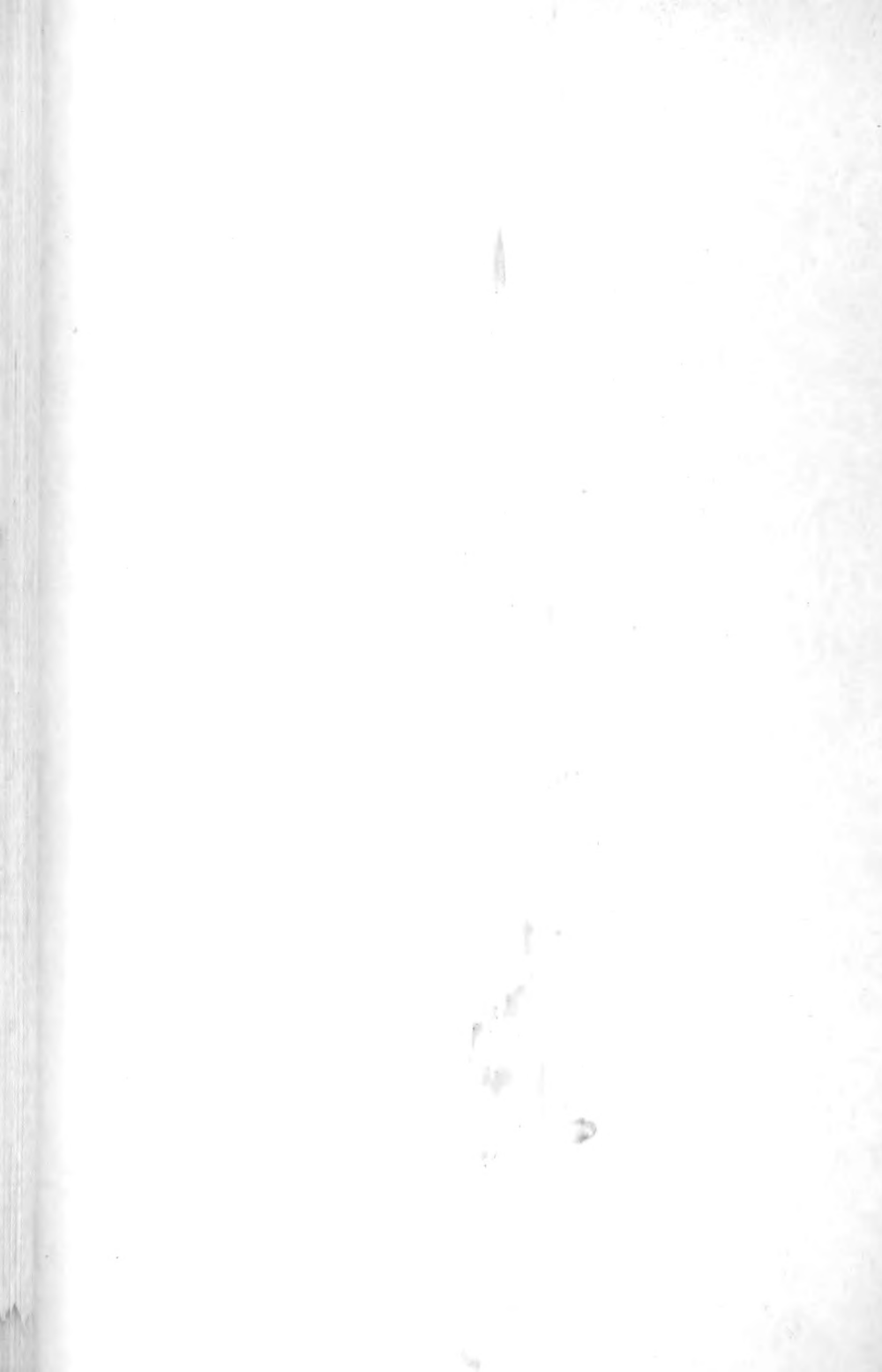
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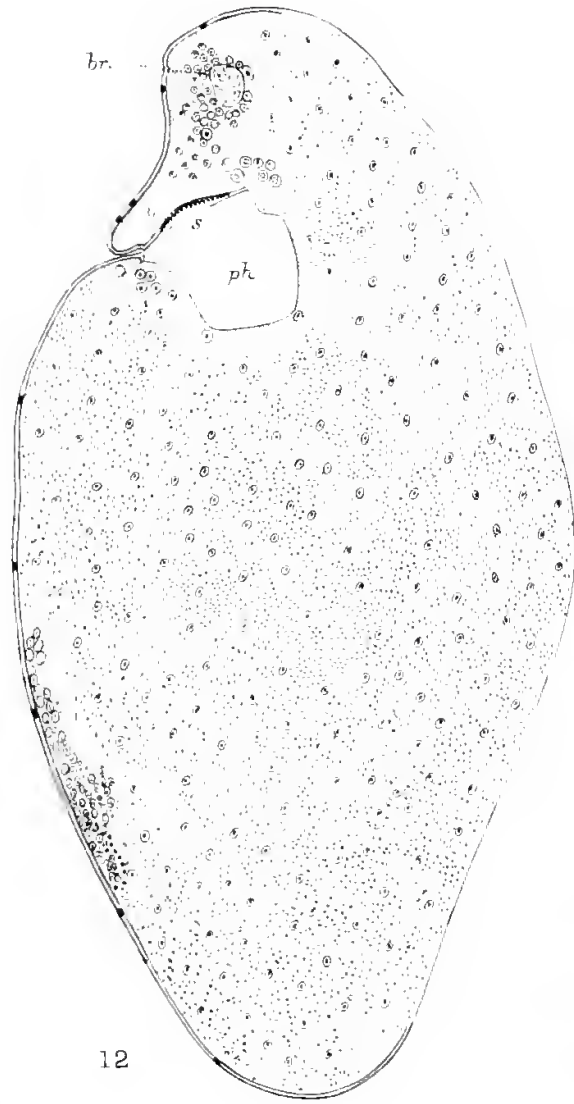
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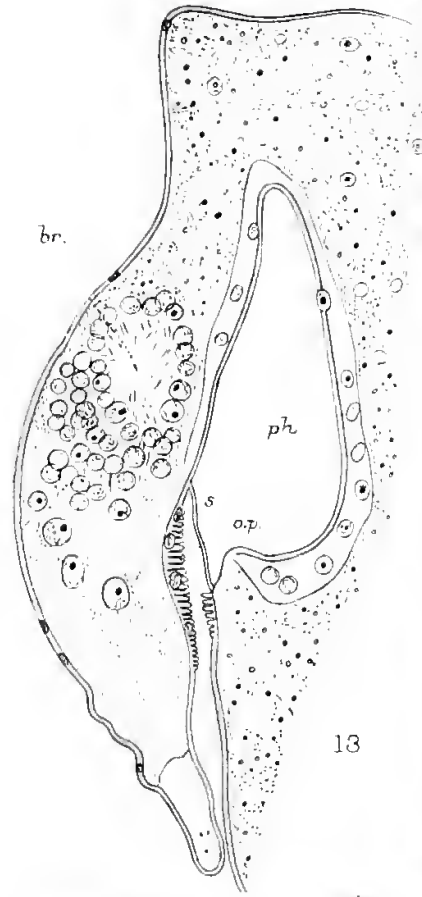
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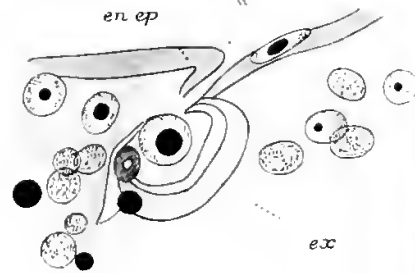




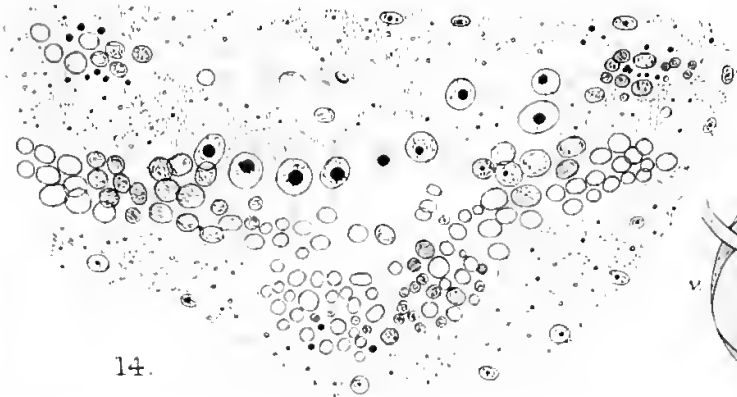
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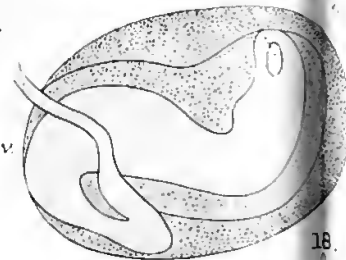
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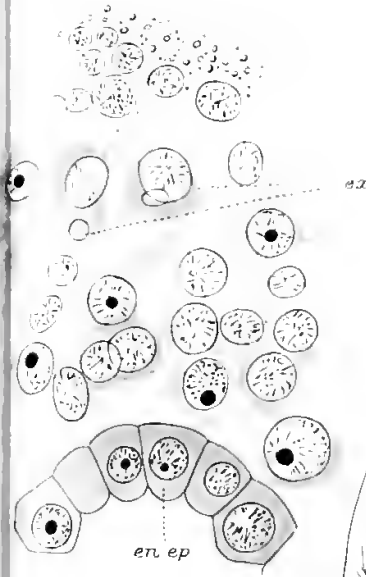
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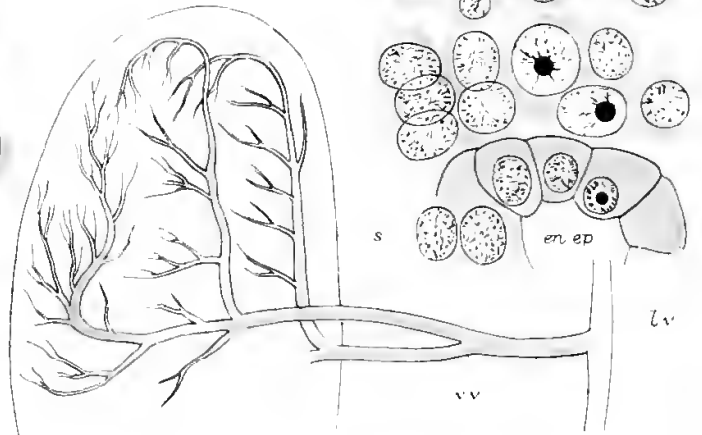
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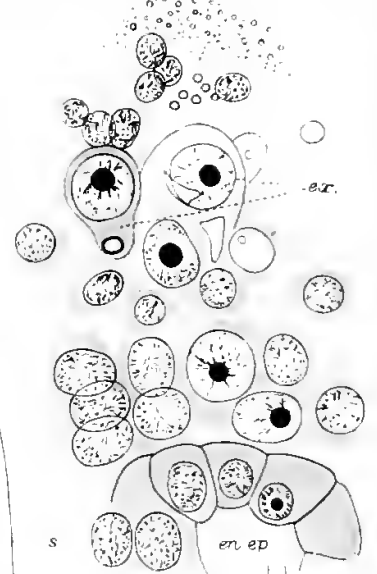
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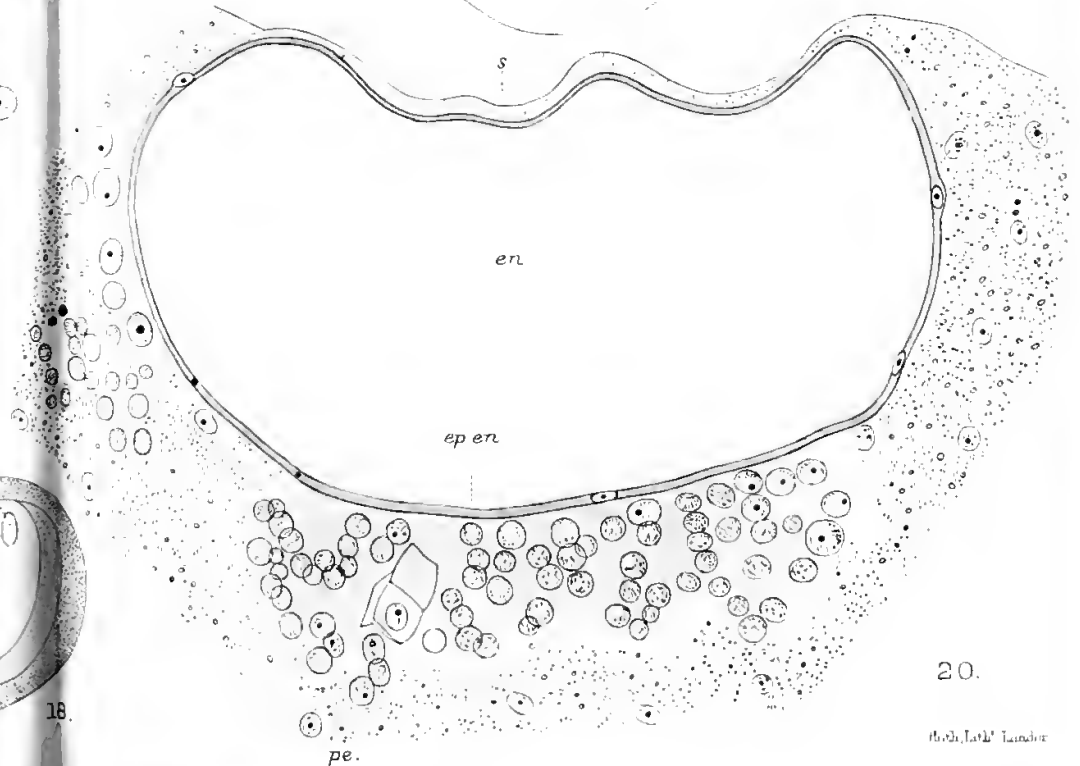
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20.

Experimental Observations on the Organs of Circulation and the Powers of Locomotion in Pennatulids.

By

Edith M. Musgrave, D.Sc. (née Pratt),
Late Honorary Research Fellow in the University of Manchester.

With Plates 26 and 27.

THE study of Pennatulids has long engaged the attention of zoological workers. In the publications of the eighteenth century wonderful and fantastic accounts are given of the habits, form and movements of members of this interesting family; of their graceful flight through the water, their exceeding luminosity by night, and their gymnastic contortions even in captivity. In those early days of zoological research a "sea pen" was regarded as a single individual provided with lateral "fins" for swimming purposes, and a mouth opening at the base, which served also for the excretion of waste matters.

Later accounts on the subject are written in a more temperate spirit, and in them we observe a general contradiction of the apparently extravagant statements of early writers.

In the tenth edition of the 'Systema Naturæ' of Linnaeus (1758) a diagnosis of *Pennatula phosphorea* is given, in which mention is made of a basal opening to the exterior (*os baseos commune rotundum*). Six years later Ellis (1764, pp. 419-428) published an excellent account of an investigation of this species, in which he denies the existence of the mouth or basal opening. Of the habits of this interesting species he says:

"It floats and swims about freely in the sea, whereas corals, corallines, Alcyonia and all that order of beings adhere firmly by their bases to submarine substances . . . This species . . . has been found in the ocean from the coast of Norway to the most remote parts of the Mediterranean Sea, and not only dragged up in trawls from great depths of the sea, but often found floating near the surface."

Dr. Shaw, in his 'History of Algiers,' remarks that they afford so great a light in the night to the fishermen that they can plainly discover the fish swimming about in various depths of the sea. He states that from this extraordinary property Linnæus called this species of sea pen "*Pennatula phosphorea*," and remarks: "Habitat in Oceano fundum illuminans."

Of the leaves or pinnæ of the sea pen Ellis says:

"These are evidently designed by Nature to move the animal backwards or forwards in the sea, consequently to do the office of fins, while at the same time the suckers (auto-zooids) or mouths furnished with filaments or claws (tentacles) were certainly intended to provide food for its support, for notwithstanding what Dr. Linnæus has said in regard to its mouth in his 'System of Nature,' viz. os baseos commune rotundum, I could not, with the aid of the best glasses, discover that the point of the base was penetrated in the least."

Bohadsch (1761) had already denied the existence of a basal mouth, and stated that the cavity or sinus, which usually exists at the base of the stalk (fig. 13), is merely a space caused by the contraction of the axis.

This assertion is supported by Ellis (p. 430), who makes the following statement:

"The bone (axis) is fastened to ligaments at both ends, which are likewise inserted in both ends of the animal. When the ligament at the base is contracted, it forms that sinus at *a. a.* (Ellis, 1764, figs. 1 and 2, pl. xx) which has been taken for a mouth by most authors."

So convincing, apparently, was the joint denial by

Bohadsch and Ellis of the existence of a mouth or of any basal opening to the exterior that the statement "*os baseos commune rotundum*" was omitted in the diagnosis of the genus in the eleventh and subsequent editions of Linnæus' '*Systema Naturæ*.'

Later, when the knowledge of the colonial habit of the "sea pens" had become established 'Ellis and Solander' (1786), in a joint publication, made the following statement:

"They have no opening at the bottom as was formerly thought, nor any other passage but through their polyp mouths"; but notwithstanding this emphatic denial O. F. Müller (1788) recorded the presence of a pore at the base of the stalk in *Kophobelemnion*, and later Della Chiaje (1827) described, with accompanying illustrations, the interesting occurrence of a basal pore in specimens of *Pterœides spinosum*, *Pterœides griseum* and *Pennatula rubra*, and furthermore stated that the pore communicates with the canals in the stalk.

These observations were confirmed by Kölliker (1872), who noticed in some cases a single pore, and in others two pores at the base of the stalk in *Pterœides* and *Pennatula*. In 1875 Kölliker's statement was further confirmed by Schultze, but Hnbrecht (1882, p. 572) was unable to observe any basal aperture in the case of the new genus *Echinoptilum*, and Marshall (1887, p. 29), who appears to have made the most recent observation on the subject, remarked that he was unable to confirm Kölliker's statement with regard to the presence in *Pennatula* of a minute orifice at the base of the stalk opening into the canals.

I have therefore sought experimental evidence, which is recorded in the present paper (pp. 452-459), in the hope of obtaining a satisfactory and final solution of this questionable point.

The early accounts of the active swimming movements of Pennatulids have been, during recent years, somewhat discredited. There can, however, be little doubt that they

exhibit, under normal conditions, certain controlled movements of the colony apart from the mere expansion and contraction of the zooids.

The earliest account of such movement was given by Lancaster¹ (1601) who, on attempting to pluck up a growing *Virgularia*, observed it to "shrink down to the ground unless held very hard."

A later account of the movements of the colony was given by Bohadsch (1761, p. 424) who makes the following statement with regard to the conduct of a living specimen of *Pennatula phosphorea* in sea-water :

"The trunk was contracted circularly at the bottom of the naked part of the stem, and by this contraction formed a zone of the most intense purple, which moved upwards and downwards successively. When it moved upwards through the length of the pinnated trunk it there became paler, and at length terminated at the top; the motion being scarce finished, a like zone appeared at the end of the naked trunk, which finished its motion in the same manner as the former."

The peristaltic dilatation and contraction thus originally described by Bohadsch has been confirmed by Kölliker, who suggests that the boring in the sand is effected by this means, but the statement also made by Bohadsch in the same publication that a specimen of *Pennatula* was seen "swimming freely with all its delicate transparent polypi expanded . . . sailing through the still and dark abyss by the delicate and synchronous pulsations of the minute fringed arms of the whole polypi" still requires confirmation, although Ellis and Solander (1786), in the diagnosis of the genus *Pennatula*, state :

"This genus of animals differs remarkably from all other zoophytes by their swimming freely in the sea, and many of them have a muscular motion as they swim along. I know of none of them that fix themselves by their base."

In his account of the *Pennatulidæ* in the 'Cambridge Natural History' (Cœlenterates, p. 360), Hickson writes :

¹ See Darwin, 'Naturalist's Voyage round the World,' p. 99, 1845.

"The sea pens are usually found on muddy or sandy sea bottoms, from a depth of a few fathoms to the greatest depth of the ocean. It is generally assumed that their normal position is one with the peduncle embedded in the mud and the rachis erect. Positive evidence of this was given by Rumphius writing in 1741 in the case of *Virgularia rumphii* and *V. juncea* at Amboina, and by Darwin in the case of *Stylatula darwinii* at Bahia Blanca. "At low water," writes Darwin, "hundreds of these zoophytes might be seen projecting like stubble, with the truncate end upwards, a few inches above the surface of the muddy sand. When touched or pulled they suddenly drew themselves in with force so as to nearly or quite disappear."

"It is not known whether the Pennatulids have the power of moving from place to place when the local conditions have become unfavourable. It is quite probable that they have this power, but the accounts given of the sea pens lying flat in the sand do not appear to be founded upon direct observation."

This prone position was, unfortunately, the usual condition of all the Pennatulids observed in captivity in the experimenting and exhibiting tanks provided with sandy or pebbly bottoms in the Naples aquarium. I am also informed by Dr. Lo Bianco, of that institution, that specimens kept thus in captivity constantly remain prone, and have never been observed to display any of those active swimming muscular movements of the leaves so frequently commented upon by early writers. It is very possible that specimens described as swimming thus through the ocean depths have become voluntarily or involuntarily removed from their basal attachments, while the flapping movements of the leaves may have been induced by water currents and other mechanical external causes. They are certainly not induced by muscular effort on the part of the individual colonies. This question is further discussed in the present paper.

Bohadsch described four different movements of the "leaves," then universally regarded as fins, thus: "They

are moved both towards the naked stem and towards the pinnated stem; sometimes they are drawn in very much to the belly, a little after they are inclined to the back."

I regret that I can produce no evidence in support of this statement. The movement of the leaves was observed in all cases to be extremely slight, and extended over a considerable interval of time. In many cases it was only after several hours that the leaves became slightly inclined ventrally or dorsally upwards or downwards, but the change in position was invariably accompanied by a change in their contour, and seemed to be entirely dependent upon the state of hydrostatic dilatation or otherwise of the colony.

Upon internal examination of the rachis, the tissue around the insertion of the leaves was observed to be composed almost entirely of that distensible spongy tissue which we have reason to suppose plays no unimportant part in the dilatations and contractions of the colony (pp. 463-464). In this region the musculature is extremely feebly developed, and serves chiefly for the protraction and retraction of the autozooids, the cœlenteric cavities of which are separated from the large canals of the rachis merely by a thin connective-tissue layer. The arrangement of the musculature in connection with the spongy tissue and controlled movements of the colony is further discussed on pp. 460-473.

THE CANAL SYSTEM.

As very excellent accounts already exist of the form, arrangement, and general anatomy of the canal system of Pennatulids it is necessary to add little to what has already been published on this portion of the subject.

For the earliest account we are indebted to K  lliker (1880), who described most carefully and thoroughly the arrangement and general anatomy of the canal systems in several genera: *Pennatula*, *Pter  ides*, *Virgularia*, etc. He also states that, with the exception of the presence of the four large central canals in Pennatulids, the canal system

is very similar to that of *Alcyonium* and *Corallium rubrum*.

The brothers Marshall (1882) in the classic memoir on the Oban Pennatulida gave admirable accounts of the canal systems of *Pennatula* and *Virgularia*, in which the observations of K  lliker are to a great extent confirmed and supplemented.

Jungersen, 1904, describes for the first time the canal system of *Protoptilum*.

Gravier, 1906, gives an account of a curious modification in the arrangement of the canals in the Virgularian genus *Scytaliopsis*.

Notwithstanding the valuable contributions which K  lliker, Marshall, Jungersen, and Gravier have made with regard to our knowledge of the general anatomy and arrangement of the canal systems of Pennatulids, their actual function still remained a matter of uncertainty. Experimental evidence has therefore been sought with the hope of obtaining some elucidation of this unknown problem.

All species of the genera *Pennatula*, *Pter  ides*, *Virgularia* and *Anthoptilum* which I have had the opportunity of examining agree in the following respects :

In the presence of the four large central canals—one dorsal, one ventral and two lateral canals. The dorsal canal¹ extends from the extreme base to the distal end of the colony, and is separated from the ventral canal by the vertical septum (figs. 12, 13, 14), and the septum terminates inferiorly by fusion ventrally with the connective tissue of the body-wall, usually, as in *Pter  ides* some little distance above the basal termination of the stalk (fig. 14), so that the extreme lower portion of the stalk is occupied only by the dorsal canal.

In *Pennatula rubra* the vertical septum extends almost but not quite to the extreme base of the stalk (fig. 13).

¹ I have used the terms "dorsal" and "ventral" in accordance with Jungersen's designation, which is the reverse of that formerly adopted by K  lliker and other antecedent writers.

The dorsal and ventral canals communicate with each other by means of numerous apertures in, or short canals which traverse the vertical septum.

The two lateral canals are considerably shorter than the dorsal and ventral canal; they also communicate with the latter at frequent intervals by means of pores in the intervening septa. In *Pterœides*, according to Kölliker, each of the two lateral canals is in direct communication inferiorly with the lumen of the dorsal canal. In all cases the cœlentera of apparently all the zooids communicate by means of more or less short canals with the four large central canals. In some cases these connecting canals are ciliated as in *Pennatula murrayi* (Hickson)¹ in others they are funnel-shaped as in *Protoptilum carpenteri* (Jungersen, 1904, p. 53).

In the stalk, which is usually destitute of zooids, the large canals communicate with the exceedingly numerous canals of the spongy tissue which line the internal body-wall of stalk and rachis, by means of numerous apertures (figs. 13, 14, *Ap.*), arranged in longitudinal lines between the insertion of the long oblique muscles controlling the movements of the axis.

In the genus *Virgnlaria*, as in other *Pennatulids*, the cœlentera of the zooids are in communication with the large central canals, but according to the brothers Marshall (1882) supplementary radial canals are also present in the rachis, which do not communicate with the cœlentera of the zooids, but run outwards towards the peripheral tissues.

In *Scytaliopsis* (Gravier, 1906) similar tubes are apparently present, but in this genus they open directly to the exterior. Gravier states that these tubes, with their external apertures, are extremely numerous, and contrasts their abundance with the paucity of zooids in this genus.

It would be extremely interesting to determine whether in

¹ Professor Hickson has drawn my attention to the presence of certain "ciliated" canals in the rachis of *Pennatula murrayi*. These canals are seen in section to be connected by means of other canals which appear to be unciliated, with the large central canals.

Virgularia the radial canals described by Marshall have communications with the exterior as in Scytaliopsis. These supplementary canals, according to Marshall, do not occur in Pennatula or Funiculina.

The occurrence of the "radial canals" in Virgularia is no doubt correlated with the paucity of the zooids. This modification has probably been brought about by the insufficiency of the zooids, to serve hydrostatic and possibly also nutritive purposes.

NEMATOCYSTS.

As a general rule the Pennatulids are less formidably armed with those weapons of offence and defence—the thread-cells or nematocysts—than the Alcyonidæ.

The tentacles are usually smooth and filamentous, and lack the characteristic warted appearance of those of Alcyonids, in which each wart is a battery of poisoning and paralysing thread-cells. Nematocysts of the usual type are present, but in considerably smaller numbers, and are more or less uniformly distributed in the general ectoderm of the tentacles.

From their constitution and habits Pennatulids would appear to be less voracious in their habits than are Alcyonids generally, and are apparently more dependent upon the supply of nutriment suspended in the sea-water in the form of organic particles, which may be carried into the zooids and canals by inflowing currents, where they are ingested and digested by the mesenterial filaments of the autozooids, as in Alcyonium (Pratt), or by any epithelial surface of the canal system (fig. 9).

THE EXPERIMENTS.

The experiments recorded in the present paper were performed in the Zoological Laboratories of the Marine Biological Station at Naples during the month of April, 1905.

I.

Several colonies of *Pennatulula rubra* and *P. phosphorea* were placed in tanks of running, filtered sea-water. At frequent intervals tiny clouds of finely powdered carmine were squirted around the pens by means of a small glass syringe. After four days the colonies were submitted to microscopic examination when particles of carmine were observed.

(1) In the cœlentera of the autozooids and siphonozooids and in an ingested condition in the cells of the mesenterial filaments of the autozooids (Pratt, Alcyonium, 1905).

(2) In the canals connecting the cœlentera of the zooids with the four large central canals of the stalk and rachis.

(3) In the lumen of the four large canals just mentioned.

(4) In an ingested condition in the cells composing the cœlenteric lining of the zooids, and also in many of the cells composing the epithelial lining of the large canals throughout the colony (figs. 1, 9).

This experiment, in my opinion, indicates the existence of a very complete and comprehensive system of communication between every portion of the colony and the exterior, while the presence of ingested particles of carmine in the cells lining the cœlentera of the zooids and the lumen of the large canals throughout the colony confirms the opinion based upon numerous feeding experiments on other members of the Order Alcyonaria (Pratt, 1905), that these cells possess at least a nutritive function (fig. 9), and indicates the possibility of the ingestion and digestion by any epithelial surface (other than that occupied by the mucus-secreting gland-cells) of any nutritive matter which may be present in the fluid circulating so freely within the colony. Of the nature of this fluid comparatively little is known, and it was, unfortunately, impossible to obtain a chemical analysis during experimental operations, but it is evident that this question was not entirely ignored even in a period so remote as the eighteenth century, for Bohadsch (1761), in the account of his investigation of living specimens of *Pennatulula phosphorea*, states:

"When the trunk is opened lengthways a saltish liquor flows out of it, so viscid as to hang down an inch."

The viscosity of the fluid contained in the canals was noticeable in all the specimens which came under my observation, and is, in my opinion, attributable to a mucoid secretion which exudes in considerable quantity from a profusion of gland-cells in the epithelial linings of the large canals (figs. 8 and 9) of the stalk and rachis. In this viscid exudation from the deeply staining gland-cells particles of carmine were often observed.

After an interval of a hundred and twenty years, during which period the zoological records are mute on this subject, Kölliker (1880) published a carefully compiled account of the canal systems of Pennatulids, but apparently makes no statement as to the nature of their fluid contents. The canals, however, he describes as "nourishing canals," so that doubtless he considered the probability of the presence of nutrient matter in the liquids contained in them.

Later the brothers Marshall (1882, p. 33) made a statement to the effect that the fluid contained in the canal system of *Pennatula* is probably nutritive but mixed largely with sea-water.

After a comparative study of the arrangement and anatomy of the canal system of Pennatulids and other Alcyonaria (*Alcyonium*, *Corallium*, etc.) and their physiological behaviour and condition when subjected to experiments with nutrient (Pratt, 1905) and non-nutrient substances, as in the experiment herewith recorded, we have strong evidence for believing the fluid contents of the canal system to be composed mainly of sea-water, in which nutrient matter may be dissolved or suspended in minute particles, and a mucoid secretion (fig. 9) in which foreign particles, harmful or useless to the colony, may become embedded and carried to the exterior.

II.

A freshly captured specimen of *Pennatula rubra* was placed, as in the former experiment, in a tank of running, filtered sea-water about 5 p.m., April 14th. By means of a

minute incision in the dorsal surface of the middle of the rachis, an injection of finely powdered particles of carmine in suspension in sea-water was made, with the aid of a small syringe, into the dorsal canal. The injected fluid passed immediately to the base of the stalk, its presence being revealed by the transparency and tenuity of the body-wall in this region (fig. 1). Three quarters of an hour later no apparent change had taken place in any other portion of the colony, but at 8.15 o'clock the following morning a thin, viscid stream containing carmine particles was observed to ooze from a single pore at the base of the stalk. Similar streams, but more minute in quantity, were also observed to proceed from four small pores also hitherto unrevealed, in the median naked streak in the dorsal surface (fig. 5) about 25 mm. from the extreme apex.

About 10 a.m. the same day the ventral canal of the same specimen was injected from the ventral surface in a similar manner with a solution of methylene blue in sea-water, when a portion of the injected fluid was immediately discharged through the mouths of the more ventrally situated siphonozoids and autozoids, while a considerable portion passed almost to the extreme base of the stalk and was discharged through an aperture which then only became apparent. Two basal apertures were thus revealed: one slightly dorsal to the other extruding particles of carmine, the second, slightly ventrally placed, discharging the solution of methylene blue. Several hours later, however, carmine and methylene blue were observed to emerge together from the ventral aperture.

The two basal apertures thus revealed were afterwards found to be direct openings of the dorsal and ventral canals to the exterior (figs. 1 and 2). The experimental evidence of their presence and active physiological function in living specimens gives an affirmative answer to the controversy which has existed since the eighteenth century (pp. 443-445) as to whether Pennatulids have or have not a basal communication with the exterior (figs. 1 and 2).

A short time after the two basal apertures became apparent, minute streams of methylene blue were observed to emerge from six additional apertures, also about the base of the stalk, four of which were definitely arranged to form the four corners of a square when viewed basally (fig. 2) and the other two laterally placed but in close proximity to the dorsal and ventral apertures. Particles of carmine were observed in a state of extrusion from one only of all the six apertures (fig. 2, *L.Ap.*).

Particles of carmine were also observed in a state of extrusion from the mouths of the autozooids and of the siphonozooids (fig. 7), but only extremely minute particles were seen to be expelled from the latter; it may have been impossible for larger particles to escape that way.

The specimen under consideration differed from all others which I have had the opportunity of examining in the presence of an aggregation of unusually large siphonozooids in the region superior to that occupied by the dorsal pores (fig. 5, *sl*₁). From these zooids I observed no instance during the experiment of the extrusion of carmine or methylene blue. It is possible that at the time of examination the injecting fluids had not then penetrated to that particular region; but it was very evident that currents were passing outwards from these siphonozooids, for clouds of methylene blue squirted among them were immediately dispersed in an outward direction.

On the third day the colony was fixed and preserved for future examination in a moderately hot 7 per cent. aqueous solution of formalin.

At intervals during the month of April this experiment was repeated on other freshly captured specimens of *Pennatula rubra*, and in three instances in specimens of the species *P. phosphorea*.

The results in all cases were very similar in character. Four dorsal pores in the act of extrusion of coloured liquids and solid carmine particles were observed in all the specimens examined of both species (fig. 5, *D.P.*)

The large basal apertures of the dorsal and ventral canals

were also present and also the four additional apertures forming the four corners of a square (fig. 4, *L.ap.*), but in the case of *P. phosphorea* the two lateral apertures in close proximity to the dorsal and ventral apertures in the species *P. rubra* were not observed.

III.

Healthy freshly captured specimens of *Pterœides spinosum* were submitted to injections similar to those already described in *Pennatula*, and as the results differed somewhat in the two forms, it will be more convenient to consider the case of this genus separately. As the body-wall, and, in fact, the texture of the whole colony in *Pterœides*, is much stouter, tougher, and more muscular than in *Pennatula*, some considerable time elapsed before a carmine injection of the dorsal canal about the middle of the rachis caused any appreciable difference in the appearance of the colony; but after an interval of more than twenty-four hours particles of carmine were extruded from four minute dorsal pores near to the apex of the rachis, which, as in *Pennatula*, only became discernible after injection. Carmine was also extruded in the form of minute particles from several pores about the base of the stalk (fig. 3); of these, one larger than the others, and almost medianly situated, had tumid lips, and proved later to be the dorsal aperture of the large dorsal canal (fig. 3A, *B.D.ap.*). Four slightly smaller apertures were also actively engaged in the extrusion of carmine.

The day following the lower end of the stalk of the same specimen (hereafter known as specimen No. 1), was placed in a solution of methylene blue in sea-water, care being exercised that the remaining portion of the colony, while submerged in clear sea-water, should not be exposed to any material extent to the influence of the coloured fluid. This was accomplished by placing the stalk into a deep, narrow-necked vessel containing the methylene blue solution, and carefully introducing

the whole into a tank of sea-water, so as to entail as little diffusion of liquids as possible.

After an interval of two hours the colony was examined in fresh sea-water, when it was found that the coloured fluid had been absorbed by a single pore only. This proved to be the basal ventral aperture of the large ventral canal (fig. 3A, *B.V.Ap.*). Carmine particles were still observed in the act of extrusion in considerable quantity from the basal, dorsal, and other apertures.

This experiment indicates the simultaneous presence of an inhalent current into the ventral canal, and several "exhalent" currents, the principal one being from the large dorsal canal to the exterior (fig. 3).

It is by no means proved that such conditions always prevail. It is very probable that under certain conditions the currents may be entirely inhalent or entirely exhalent; the direction of the current is doubtless dependent upon the individual requirements of the colony and responsive to stimuli from within. This matter is further discussed on pp. 470 and 471.

The stalk of a second specimen of *Pterœides spinosum* was placed in a narrow-necked vessel as before, but the vessel contained in this instance finely powdered particles of carmine. After several hours had elapsed the stalk of the colony was opened and examined, but no trace of carmine was observed in any of the canals. The currents during the experiment may have been entirely exhalent, but there seems some evidence for the supposition that the colony has the power to some extent of filtering the sea-water as it enters the canals basally. We have reason to believe that such is not always the case, for in other Pennatulids particles of earthy foreign matter are frequently observed in the large canals near the base of the stalk (cf. *Pennatula rubra*, as indicated in Plate 26, fig. 1).

On the third day specimen No. 1, which still remained apparently healthy and responsive to stimulation, was injected with a solution of methylene blue from the ventral canal about

the middle of the rachis. As in the case of *Pennatula* the coloured solution was immediately ejected in continuous streams from the autozooids and siphonozooids, indicating a very brisk circulation of fluid within the colony, the direction of the currents being mainly exhalent.

Within a very few minutes the methylene blue streamed from several basal pores which then became evident. These pores had taken no part previously in the expulsion of the solid particles of carmine. Several of the pores are indicated in fig. 3.

In all cases the injection of a coloured fluid into the dorsal canal was immediately followed by its distribution to all parts of the colony and expulsion from autozooids and siphonozooids, and, indeed, after a very few minutes from the basal and dorsal pores.

Upon examining a dilatation in the region of the stalk, which appeared in a specimen of *Pterœides* shortly after an injection had been made, the coloured fluid was found in considerable quantity in the "spongy tissue" of this particular region of the colony, giving evidence in support of the supposition of its distensibility, in assisting in the dilatation and contraction of the colony (pp. 463, 464).

These facts afford an interesting illustration of the extreme rapidity and comprehensiveness of the circulation of fluids within the colony under what appear to be normal conditions.

In the case of the injection into the dorsal canal of the rachis of powdered carmine suspended in sea-water, the distribution of these solid but minute particles proceeded much more slowly. In the case of *Pennatula* the injecting fluid was transferred immediately to the base of the stalk, but the subsequent ejection of carmine particles did not take place from autozooids or siphonozooids until several hours had elapsed, and after a still further interval from the basal apertures and dorsal pores.

In *Pterœides* the interval between the injection and extrusion of carmine extended over twenty-four hours. Its ingestion in minute quantities and subsequent protrusion from the cells lining the canals may to a great extent be

responsible for the delayed progression. The metabolic influence of the contents of the circulating fluids upon the tissues bathed by them is not without interest to the physiologist.

During fixing operations the contour of the colony and especially that of the stalk usually changed somewhat.

This was particularly noticeable in the case of *Pterœides*, in which the fixative re-agent penetrated the thick and tough body-walls very slowly, allowing the internal axis to become considerably shortened by muscular contractions, and bringing about a slight invagination of the extreme base of the stalk. The normal basal apertures were thus completely obliterated, but the slight invagination of the body-wall gave the appearance of a single aperture, which is so usual in preserved specimens of Pennatulids that it has been frequently described as a mouth opening by early writers (fig. 3B), (Linnæus, Della Chiaje, etc.), and as frequently denied by later ones (Marshall, Pennatula; Hubrecht, Echinoptilum).

In the case of *Pennatula* it was possible to fix and preserve specimens in which the two apertures of the dorsal and ventral canals were discernible, but in *Pterœides* and other forms all the basal and dorsal pores were obliterated during the operations of fixing and preserving.

The peristaltic dilatations and contractions described by Bohadsch and Kölliker were observed in the case of *Pterœides* and *Pennatula*, but in all cases the movements were confined to the stalk and rachis, and were somewhat lethargic even in freshly captured and apparently healthy specimens, in some instances being so slight as to be barely perceptible.

There can be no doubt that specimens examined in captivity suffer considerably in this respect from their change of environment, but a study of the internal anatomy of the stalk and rachis of *Pterœides*, *Anthoptilum*, *Virgularia*, and *Pennatula* furnishes indisputable evidence at least of the muscular activity of the axis as a boring organ, and suggests also the probability of its function as a propelling organ in these genera.

THE MUSCULATURE OF THE STALK AND RACHIS.

The musculature of the "body-wall" of Pennatulids has been carefully worked out by Kölliker (1872) and the brothers Marshall (1882). For descriptive accounts, therefore, the reader is referred to these authorities.

In *Pterœides* the body-wall is generally dense, opaque, and of tough, fibrous texture. In the specimens of this genus the musculature is correspondingly more strongly developed than in those of *Virgularia*, *Anthoptilum* and *Pennatula*. In the species "*phosphorea*" of the last-named genus an extreme condition in the opposite direction is attained, for in this form the body-wall is very feebly developed, and the stalk is characterised by its delicacy, fragility, and transparency. The musculature is, in accordance, composed of less numerous and more delicate fibres.

In all cases the body-wall of the stalk contains two series of muscles: an "outer layer of longitudinal muscles," and an inner layer of transverse and frequently circular muscles.

The "longitudinal muscles" lie immediately below the dermis, and are composed of comparatively long muscle-fibres whose direction is parallel with the longitudinal axis of the colony. These muscles form a continuous layer in the stalk, and pass upwards into the dorsal and ventral portions of the rachis, but are discontinued laterally, the musculature of the sides of the rachis, and particularly at the insertion of the leaves and pinnæ, being so feebly developed as to serve only for the protraction and retraction of the zooids, and can take no part whatsoever in the movements of the leaves. We have absolutely no evidence for the statements made by Ellis, Bohadsch, and others, of the muscular swimming movements of the leaves or "fins," as they were formerly termed.

The longitudinal muscles of the body-wall assist in the support of the colony by strengthening the tissues of the

body-wall, and are also actively concerned in the extension and contraction of the colony in a vertical direction, and thereby assist in directing the circulating currents within the canals.

The "transverse muscles" of the body-wall are more deeply seated than the longitudinal muscles, and usually lie on the inner side of the spongy tissue. In the stalk their general direction is usually at right angles to the longitudinal axis of the colony, and in this region the muscles are usually arranged in more or less complete rings. In the rachis the fibres are less definitely arranged and are also less numerous. These muscles materially assist in the support of the colony by strengthening the central axis, but their main function is apparently that of instituting and maintaining, by peristaltic action, a series of dilatations and contractions of the stalk and to a less degree of the rachis, thereby assisting in controlling the quantity of fluids contained in the canals and also in directing the circulating currents.

Marshall believed the circulation of fluids within the colony to be largely dependent upon the muscular system of the body-wall. He wrote of *Pennatula phosphorea*: "The well developed muscular system (i. e. of the body-wall) may be supposed to have for its main function the maintaining, by compression of portions of the spongy mesh-work, of currents from one part of the pen to another." As this system of muscles appears to be more feebly developed in this than in any of the other forms under discussion, Marshall's statement would apply with greater force to *Pennatula rubra*, *Anthoptilium*, *Virgularia*, and with special emphasis to *Pterœides*. We have, however, evidence for believing that, with the exception of the "axis-less" form *Renilla* (p. 462), the musculature of the body-wall is not the sole force in inducing, maintaining, and controlling the circulating currents.

Wilson observed certain creeping movements of living specimens of *Renilla*—a Pennatulid without a central calcareous axis—which he attributed to the movement of fluids

within the "gastric cavity."¹ Of this interesting Pennatulid he writes: "The anterior part of the body being well distended, an active peristaltic contraction of the circular muscles takes place, and the fluid is thus forced backwards into the posterior region (peduncle). The latter consequently becomes elongated, somewhat as the ambulacral 'foot' of an echinoderm is protruded, and the body is pushed forward a short distance. The circular muscles then relax and the longitudinal ones contract in such a manner as to pull the posterior region forward towards the anterior part which adheres to the bottom. By the constant repetition of this process the whole organism moves slowly forwards. The creeping movements are very irregular since the action of the muscles is not uniform."

In large and robust Pennatulids having a central, more or less rigid, calcareous axis, certain modifications of structure have arisen which apparently play an important part in inducing and maintaining a comprehensive circulation of fluids throughout the colony.

In all the genera examined the musculature of the body-wall becomes attenuated towards the base of the stalk (figs. 12, 13, 14), its place being occupied by the spongy distensible tissue which is so abundantly provided with apertures into the large canals, and also with a definite number of apertures (which appears to be constant on each species) to the exterior (figs. 1, 2, 3, 4). In living specimens this region is often considerably dilated by the absorption of fluids by the canals of the spongy tissue, so that the stalk terminates basally in a small distended bulb, which, if buried in the mud of the sea bottom, may materially assist in supporting the colony in a vertical position.

The distension may also represent the initial stage of the peristaltic movements of the stalk and rachis commented upon elsewhere (p. 473).

¹ By the term "gastric cavity" Wilson, no doubt, means the spaces occupied by the large central canals of the stalk and rachis.

THE SPONGY TISSUE.

The spongy tissue varies considerably in consistency among the members of the Pennatulids examined.

In all the specimens of *Pterœides* it is extremely dense and tough; in *Pennatula phosphorea* it is slightly transparent and delicate in texture, while specimens of *Pennatula rubra*, *Anthoptilum*, and *Virgularia* represent an intermediate condition in these and other respects. In all cases the spongy tissue appears upon microscopic examination to be made up of a more or less homogeneous deeply staining matrix, traversed in all directions by numerous canals having epithelial linings. They communicate by means of numerous apertures—usually arranged in longitudinal rows (figs. 13, 14, *ap.*), with the large dorsal and ventral canals of the stalk and rachis, and at the base with the exterior by means of a definite number of pores which appear to be constant for any given species (figs. 1, 2, 3, 4).

The "spongy tissue" is capable of becoming rigid and turgid by the absorption of liquids by its canals. On occasion these may be filled to distension, or the reverse, by the combined peristaltic action of the transverse and the longitudinal muscles of the body-wall.

The "epithelium" lining the small canals of the "spongy tissue" is somewhat different from that lining the large canals of the stalk and rachis in that its cells are more cubical in shape. This tissue is apparently characterised by an absence of the deeply staining mucus, the vacuolated, and the food-ingesting cells which form the main portion of the epithelium of the large canals.

Kölliker (1870, p. 10) regarded the small canals of the spongy tissue as "nutritive canals." We have experimental evidence of the possession of a nutritive function on the part of the "epithelium" lining the large canals, but it is extremely probable that the small canals of the "spongy tissue" are developed primarily for distension purposes.

STRUCTURAL MODIFICATIONS IN THE STALK.

Several extremely large and robust specimens of *Anthoptilum grandiflorum* present a peculiar modification of the upper portion of the stalk in the form of a curiously folded and thickened, ring-like zone (fig. 10, *T.Z.*). This was found upon internal investigation to be caused by an extraordinary growth of the spongy tissue in this region (fig. 11, *L.M.P.*) in the form of numerous laminate vertical processes with free edges directed inwards, and lined internally by a moderately well-defined musculature comparable to the transverse musculature of the body-wall in other forms, but in this instance produced into the folds and convolutions caused by the remarkable development of the spongy tissue.

The laminae are traversed by numerous canals, which ramify within their substance and communicate by numerous apertures with the interlaminate interstices of the body cavity.

It was unfortunate that the state of preservation of the specimens would not permit a detailed investigation of the histology of the laminae, but a microscopic study of the body-wall in the lower region of the thickened zone proved of great interest. Its outer surface was observed to be studded with numerous minute papillae, which proved upon examination to be siphonozooids (fig. 10, *si.*)—extremely small, but of the normal Pennatulid type, with mouth apertures and the usual number of mesenteries. Their coelentera communicate as in other forms with the large central canals by means of the connecting canals of the spongy tissue.

The curiously folded epithelium of the outer body-wall, and also that of the siphonozooids, contained numerous deeply staining gland cells from which a mucus-like secretion was seen exuding in considerable quantity.

The occurrence of siphonozooids¹ in this unusual region

¹ The presence of siphonozooids in the stalk of Pennatulids is not quite unique, for Hickson (1907, p. 13) has also observed them in the stalk of "*Umbellula carpenteri*."

is of great interest, for it confirms the supposition that these modifications of the stalk in this extremely large Pennatulid have arisen for hydrostatic purposes. The curiously folded appearance of the tissue composing the thickened zone suggests the possibility during life of enormous dilatations of the stalk in this region, while the presence of the musculature on the inner surface of the laminae gives evidence of its function as an extremely powerful pumping apparatus. By its operations currents could be driven upwards with considerable force into the rachis and thence distributed throughout the colony.

In the extremely large and robust species of *Pennatula*—*P. naresi* and *P. borealis*, a slight thickening and distension of the body-wall was observed in the stalk, in the region corresponding to the folded and thickened zone in *Anthoptilum* (fig. 10). This was found to be due to a slightly increased development of the "spongy tissue" and "transverse musculature" of the body-wall in this region.

It is evident that in the case of these enormous species greater force would be required to induce and maintain the vertical currents from the stalk to the rachis than would be the case in more delicate and smaller species of *Pennatula*, e.g. *P. rubra* and *P. phosphorea*. This modification has probably arisen in the species "*naresi*" and "*borealis*" in response to the increased demands of the colony in this respect. In the presence of the slightly thickened zone in the stalk these species represent an intermediate condition between the comparatively small forms *P. rubra* and *P. phosphorea* and the more magnificent *Anthoptilum grandiflorum*.

THE SPHINCTER MUSCLE.

In the genus *Pterœides* the modification has proceeded still further. As in the forms just considered, the upper portion of the stalk in all species presents a pronounced distension, due in this instance to the development of an

internal transverse musculature, which assumes the form of an extremely powerful "sphincter muscle" (fig. 14, *Sph.M.*), whose dense and numerous fibres are attached internally to the integument of the axis and externally to the body-wall, where a slightly increased development of the musculature has arisen in that region to support this extraordinary development of muscular tissue.

Between the attachment of the muscular fibres to the body-wall are the numerous apertures (*Ap.*) of the canals of the spongy tissue.

The chief function of the "Sphincter muscles" is obviously that of dilating and contracting the diameter of the stalk in the region immediately inferior to the rachis, and thereby opening and closing the lumen of the canals. It may therefore be regarded as a very important factor in instituting and maintaining the circulation of fluids within the colony. This matter is further discussed on p. 473.

It apparently also serves as a double fulcrum in supporting the axis in a vertical position (p. 468), and assists also in the support of the "oblique musculature" of the stalk, with which it doubtless acts in conjunction (fig. 14), p. 470.

THE AXIS.

The axis of Pennatulids is usually mainly calcareous; but in Pterœides it is not wholly so, but contains in addition a considerable proportion of dense, hard, horn-like matter, which, owing to its insolubility in acid and other solutions, renders the cutting of satisfactory sections an extremely difficult and patience-exhausting task. In all cases the axis is enveloped in a sheath-like integument (figs. 11, 12, 13), delicate and membranous in smaller forms (e.g. *Pennatula phosphorea*), but usually extremely tough and of considerable density in robust types (*Anthoptilum* and *Pterœides*).

The axis varies according to the magnitude and density of the colony in length, breadth and thickness. In *Pennatula phosphorea* it is remarkable for its exceeding tenuity. In

this species it is usually thickest at junction of the stalk and rachis, where it is quadrangular in cross section, and tapers gradually above and below, becoming cylindrical as it proceeds upwards and downwards. It usually extends almost to the base of the stalk, but terminates in the rachis some little distance below the apex of the colony. It is rigid in the middle but extremely flexible at each end; and in the contracted condition of preserved specimens the basal end is usually bent upwards to form a hook as in the drawing of *Pennatula rubra* (fig. 13). The apical portion of the axis is also usually hooked when the colony is contracted, but to a less degree.

In the specimens of *Virgularia* the axis in the stalk becomes extremely attenuated towards the base, tapering gradually to a fine needle-like point, as in fig. 12. In this specimen, however, the axis does not extend to the extreme base of the stalk, as, according to the accounts given by Marshall and others, is usually the case in this genus. The unusual length of the stalk, its extreme tenuity and flexibility of the lower portion of the axis, may account to a considerable degree for the extraordinary powers of contractility for which this genus is remarkable.

The axis of *Pennatula rubra* is larger and stronger than that of the more delicate species *P. phosphorea*, and the investing integument is correspondingly stronger and thicker.

In *Pterœides* and other robust forms the axis is correspondingly stout and strong (figs. 11 and 13), and is less pointed above and below. In a state of complete extension the axis is continued from the base to the extreme tip of the colony. It is rigid throughout the greater portion of its length, but in a state of contraction it is shortened by the two flexible ends becoming hooked and slightly twisted spirally (figs. 14 and 15), the hook being more pronounced at the basal end. The spiral twist of the two ends of the axis was also observed in a dissection of *Pennatula phosphorea*.

THE MUSCULATURE OF THE AXIS.

In all Pennatulids possessing a calcareous axis, with apparently the exception of *Virgularia*,¹ in which the apical portion of the rachis is generally missing in captured specimens, the axis is supported and its movements manipulated by two series of muscles:

The apical musculature in the rachis, which supports the upper and apical portions of the axis, and the oblique musculature in the stalk, which supports the lower and basal portions.

The concerted action of the two series of muscles would extend and contract the axis superiorly and inferiorly. Their probable function is discussed later.

In *Pterœides* the axis is further supported by the powerful "sphincter muscle" of the stalk (fig. 14, *Sph.M.*) and by two strong muscle-bands to which are attached certain fibres of the inferior portion of the sphincter muscle, and numerous strong muscle-fibres of the superior portion of the oblique musculature (fig. 14, *M.B.*).

The apical musculature of the axis is most powerfully developed in *Pterœides* (fig. 15), in which the whole of the apical portion of the axis is enveloped in a muscular sheath, from which proceed numerous stout and tough fibres which run obliquely downwards and are attached to the tissues lining the body-wall. In the retracted condition they are lightly twisted in a spiral (fig. 15). This spiral twist was also indicated, but to a less degree in a dissection of *Pennatula phosphorea*.

The chief function of this musculature is obviously that of

¹ This curious and apparently general mutilation of specimens of *Virgularia*, so frequently commented upon by writers, is believed by Jungersen to be due to natural causes, and not, as was generally believed, to accident. He attributes the absence of the upper leaves and apical portion of the rachis to a gradual dying off and atrophy of the older apical portion of the colony.

supporting, extending and contracting the flexible apical portion of the axis. By the co-operation of these muscles with the longitudinal muscles of the body-wall the length of rachis would be materially increased or diminished.

The somewhat oblique insertion of the apical muscles, coupled with the action of the transverse muscles of the body-wall, although less well developed in this region than in the stalk, would nevertheless induce a slight muscular dilatation and contraction of the central canals of the rachis.

The spiral twisting of the apical portion of the axis and its ensheathing musculature, so noticeable in *Pterœides* (fig. 15) in a state of contraction, may possibly serve the purpose of a spring, which when released by the protraction of the muscles would tend to propel the colony in an upward vertical direction or vice versâ.

The oblique musculature is extremely well developed in *Pterœides* and other large and robust Pennatulids (fig. 14, *Ob.M.*).

It consists of a series of "obliquely set muscles-fibres" whose direction is mainly longitudinal. They are attached internally to the integument of the axis in such a manner that its basal portion is completely enclosed by a muscular sheath (figs. 11, 12, 13, 14), and are attached externally to the tissues lining the body-wall.

Many of these muscles-fibres are embedded throughout the greater portion of their course in the tissues of the vertical septum. In *Pterœides* the fibres are extremely stout and strong (fig. 14, *M.A.A.*). Those of the upper portion of the musculature are attached superiorly to the extremely stout, obliquely set "muscle bands" (fig. 14, *M.B.*) situated immediately below the sphincter muscle, with which it is also connected by obliquely set fibres. The muscle-bands are attached internally to the integument of the axis and to the tissues lining the body-wall; the plane of their direction is slightly inclined to the horizontal.

In the presence of the powerful "sphincter muscle and the two stout obliquely set muscle-bands" sup-

porting the oblique musculature of the stalk *Pterœides* appears to be unique.

The "oblique musculature" of the stalk is present in all the forms examined, but its development varies considerably among the members of the family. It is more powerfully developed in *Pterœides* than in any other genus (fig. 14, and apparently most feebly developed in *Pennatula phosphorea*. The specimens of *Anthoptilum grandiflorum*, *Virgularia juncea*, and *Pennatula rubra* (figs. 11, 12, 13) in which the oblique musculature is very similar, indicate an intermediate condition between these two extreme forms.

The "sphincter muscle," so pronounced a feature in the stalk of *Pterœides* and the well-marked "muscle-bands" (fig. 14, *Sph.M.* and *M.B.*) are absent in the other forms, but in *Virgularia* the oblique muscle-fibres gradually assume a horizontal inclination as they ascend the stalk (fig. 12, *M.A.A.*). While the chief function of the oblique musculature of the stalk appears to be that of supporting and controlling the movements of the inferior and basal portion of the axis, its protraction and retraction would apparently have a manifold purpose.

With the co-operated action of the longitudinal muscles of the body-wall, the protraction of the oblique muscles would straighten out the hooked ends until the axis assumed a vertical position, and the length of the stalk would thereby be increased.

The oblique action of these muscles, simultaneously assisted by the ringed transverse musculature of the body-wall, would also increase the diameter of the stalk.

The extension of the stalk in length and diameter thus accomplished would be accompanied by extensions in the same direction of the four large central canals, whose fluid capacity would thereby be considerably increased. To maintain the internal equilibrium of the colony an inhalent current would therefore be induced through the basal pores of the stalk so as to fill to distension the large central canals of the

stalk.¹ Through the innumerable apertures in the walls of the canals currents could be directed into the distensible spongy tissue (figs. 13, 14, *Ap.*). By this means the lower portion of the stalk would become considerably dilated.

The concerted retraction of these muscles would be accompanied by a simultaneous shortening of the stalk, and the hooking up of the axis in its muscular sheath in the dorsal canal would give an upward impetus with a piston-like movement to the fluids contained in it; simultaneously the retraction of the oblique muscles embedded in the vertical septum would shorten the ventral and lateral canals, which would increase the basal impetus already mentioned of the fluid in an upward vertical direction. This upward current would be maintained by the peristaltic action of the musculature of the body-wall of the stalk and to a less degree in the rachis.

In accordance with the needs of the colony the direction of the current may be reversed, as in the observations by Wilson (1893) in the case of *Renilla*.

In an experiment on *Pterœides spinosum* (fig. 3) an inhalent current was observed to enter the basal pore of the ventral canal, while several exhalent currents were observed to issue simultaneously from the pores of the dorsal and other canals in the neighbourhood of the base of the stalk. In this instance, however, the colony appeared to be in a perfectly quiescent condition, and during the experiment exhibited none of the muscular movements observed in other forms.

In the case of small and comparatively delicate species of Pennatulids (e. g. *Pennatula rubra* and *P. phosphorea*) the concerted action of the apical musculature of the rachis, the oblique musculature of the stalk, and the longitudinal and transverse musculature of the body-wall in stalk and rachis are apparently sufficient to induce and maintain a complete circulation of fluids throughout the colony.

¹ In the case of *Anthoptilum* the canals of the stalk would be rapidly filled by inhalent currents through the siphonozooids specially developed in that region.

In extremely large and robust Pennatulids such means are apparently insufficient for that purpose. In *Anthoptilum grandiflorum* (figs. 10 and 11) we find a modification of the upper portion of the stalk in preserved specimens in the form of a thickened and much-folded zone, caused by an extraordinary development of the distensile spongy tissue and transverse musculature of the body-wall. This region is also characterised by the unusual presence of numerous siphonozooids (fig. 10, *si.*).

This structural modification doubtless serves as a powerful pumping apparatus for the institution and maintenance of currents to higher altitudes and more distant parts of the colony.

An intermediate condition between the small species of *Pennatula* (*P. rubra* and *P. phosphorea*) and the magnificent species *Anthoptilum grandiflorum* is indicated in the comparatively large species of *Pennatula* (*P. naresi* and *P. borealis*). In these forms a slight protuberance of the body-wall occurs in the region of the thickened zone, and in this instance also is due to an increased growth, but to a less degree than in *Anthoptilum* of the distensile spongy tissue and transverse musculature of the body-wall.

In *Pterœides* structural modifications of the stalk, to serve the same purpose but undoubtedly with greater efficiency, has proceeded still further. This genus is characterised by the development in the stalk of an extremely powerful sphincter muscle (fig. 14), whose pump-like actions, co-operated with those of the powerfully built "oblique musculature" of the stalk and the musculature of the body-wall are apparently necessary to institute and complete the circulation of fluids within the large, densely built, and compact colony.

We have incontrovertible evidence of the distensile function of autozooids and siphonozooids in inducing inhalent and exhalent currents in the region of the rachis, and we have also experimental evidence of the function of the four dorsal pores for exhalent, and probably also for inhalent, purposes (figs. 5 and 6), but from the evidence based upon a study of the musculature in connection with the canal systems of

Pennatulids it seems extremely probable that the main impetus of the circulating circuit has its origin in the stalk.

THE POWERS OF LOCOMOTION.

It is generally assumed that the usual position of a living Pennatulid is an erect one with the stalk buried in the mud of the sea bottom. Such is undoubtedly the case with forms inhabiting shallow water like *Virgularia*, but we have reason to believe from records of observation by early writers (Bohadsch, Ellis, etc.) and from a study of the general anatomy of the Pennatulids, that the stationary erect condition is not necessarily always maintained.

It is extremely probable that many deep-sea Pennatulids are endowed with certain controlled powers of locomotion, and may transfer themselves from place to place under suitable conditions.

In *Pterœides*, for example, the protraction of the "oblique musculature" of the axis would enable the stalk with a spiral twist to bore into the sand or mud of the sea bottom in true screw-like fashion, when the bulb-like dilatation of the base would assist in supporting the colony in a vertical position. In this erect position the basal pores of the stalk (fig. 3) would, it is assumed, remain closed and inactive, and the circulatory current would now be dependent upon the activity of the autozooids, siphonozooids, and dorsal pores, for in this position the remaining portion of the colony must necessarily remain more or less quiescent, for it is obvious that any activity on the part of the muscles would dislodge it from its basal burrow.

The retraction of the oblique musculature with the co-operative activity of the apical and sphincter muscles, combined with the peristaltic action of the musculature of the body-wall would not only withdraw the colony from its basal support, but propel it with such considerable force through the water as to accomplish a swimming excursion of no small magnitude. These muscular movements which

have their origin in the stalk and rachis have doubtless been observed by early writers, and have given foundation for the belief that Pennatulids have been observed "to swim freely by means of their 'fins,'" although the "fins" or "leaves," from their very constitution cannot possibly take any part in the muscular activity and propulsion of the colony.

This publication is the outcome of certain experiments attempted on living Pennatulids in the Zoological Laboratories of the Marine Biological Station at Naples during the month of April, 1905, during which period I occupied the British Association table in that institution. I must express my indebtedness to the committee of the British Association for the grant of the table.

The greater portion of the research in connection with this paper has been accomplished in the Zoological Laboratories of the Victoria University of Manchester. I must express my very cordial thanks to Professor Hickson for much valuable information on the subject of Pennatulids and assistance in my work.

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EXPLANATION OF PLATES 26 AND 27,

Illustrating Edith M. Musgrave's paper on "Experimental Observations on the Organs of Circulation and the Power of Locomotion in Pennatulids."

ABBREVIATIONS.

Ap. Aperture. *Ax.* Axis. *Ax.S.* Axial sheath. *B.D.Ap.* Basal dorsal aperture. *B.V.Ap.* Basal ventral aperture. *B.W.* Body-wall. *C.* and *Ca.* Canals. *D.* Dermis. *D.Ap.* Aperture leading from dorsal canal to exterior. *D.C.* Dorsal canal. *D.M.S.* Delicate muscular sheath encircling the stalk near the base. *D.P.* Dorsal pores. *Ep.* Epidermis. *Ex.C.* Exhalent current. *D.S.T.* Vacuolated tissue with deeply staining cells. *F.Ap.* False aperture often seen in preserved specimens. *G.Ax.* Growing tip of axis. *In.C.* Inhalent current. *L.* Polyp bearing leaf. *L.Ap.* Lateral aperture near the base of the stalk. *L.C.* Lateral canal. *L.M.* Longitudinal muscles. *L.M.P.* Laminate muscular processes. *M.* Brownish earth-like matter on dorso-ventral septum. *M.A.A.* Muscular apparatus controlling movements of axis. *M.B.* Muscle-bands. *Mg.* Mesogloea. *M.S.Ax.* Muscular sheath encircling the axis near the apex of the rachis. *Muc.C.* Mucus cells. *N.D.S.* Naked dorsal streak. *Ob.M.* Oblique musculature supported by dorso-ventral septum. *R.* Rachis. *S.C.C.* Small canals connecting dorsal and ventral canals. *Si.* Siphonozoids. *Sp.* Spicule. *Sph.M.* Sphincter muscle regulating dilatation and contraction of stalk. *Sp.T.* Spongy tissue. *St.* Stalk. *T.Ax.* Termination of axis. *Th.B.W.* Ring-like thickening of the body-wall in the region of the insertion of the oblique muscles. *T.Z.* Thickened zone. *T.Zo.* Terminal zooid. *V.Ap.* Aperture from ventral canal to exterior. *V.C.* Ventral canal. *Y.L.* Young leaves. *V.S.* Vertical septum. *Y.Si.* Young siphonozoids.

PLATE 26.

Figs. 1-9

Fig. 1.—*Pennatula rubra*; $\times 8$. Basal portion of the stalk with cut edge as transverse section. While living the specimen had been injected in the middle of the dorsal region of the rachis with finely powdered particles of carmine in suspension in sea-water and later by a second injection of methylene blue in solution in sea-water. The deposition of carmine particles on the walls and in the lumen of the canals is shown. The distribution of the methylene blue solution in

this portion of the colony is also seen and also its extrusion from the basal apertures (*B.D.Ap.* and *B.V.Ap.*) of the dorsal and ventral canals. The brownish mass (*M.*) of earth-like matter, which appears to be deposited on the vertical septum, may represent an aggregation of foreign matter sucked in by the sea pen, or waste matter on the point of extrusion. In this genus the ventral canal extends to the base of the stalk. The four canals of the stalk are shown in cross-section. The dorsal canal (*D.C.*) is the largest, and contains in this region the two smaller lateral canals (*L.C.*), between which the basal end of the axis (*Ax.*) is shown by transparency of the walls of the canals.

Fig. 2.—*Pennatula rubra*. Basal vein of the termination of the stalk after injection with carmine particles and a solution of methylene blue as in fig. 1; $\times 7$. The two large apertures (*B.D.Ap.* and *B.V.Ap.*) of the dorsal and ventral canals are shown. Six other apertures are also indicated, which only became perceptible after injection. Of these, two are in close proximity to the large dorsal and ventral apertures and the other four definitely arranged to form the four corners of a square (*Ap.*_{1 2 3 4}). The solid particles were expelled from the large apertures of the dorsal and ventral canals, and the methylene blue solution from the other six; occasionally solid particles and coloured liquid were expelled from a common pore (*L.Ap.*₁).

Fig. 3.—*Pterœides spinosum*. (a) Drawing of the basal portion of the stalk from the left side of a living specimen twenty-four hours after an injection of carmine and methylene blue, as in fig. 1; $\times 3$. Numerous apertures are indicated which before the experiment were imperceptible to the naked eye, from which issued streams of methylene blue solution, indicated in the drawing. Carmine particles are also shown in a state of extrusion. The arrows indicate the direction of the currents. An inhalent current into the ventral canal was revealed by a prolonged immersion of the base of the stalk in methylene blue solution. It is possible that the direction of the currents may be reversed or otherwise on occasion. (b) The base of the same colony after fixing with formalin solution. The contraction of the axial tissues has caused an invagination of the basal portion of the stalk, giving the appearance of a single pore at the base (*F.Ap.*). This appears to be the usual condition in preserved specimens, and the apparent presence of a single pore has given rise to considerable difference of opinion in the past as to the presence or otherwise of a so-called mouth in this region.

Fig. 4.—*Pennatula phosphorea*. Drawing of the base of the stalk showing inhalent and exhalent apertures; $\times 30$. The two apertures in close proximity to the basal, dorsal and ventral apertures in the species "*rubra*" were not observed in this species.

Fig. 5.—*Pennatula rubra*. Drawing of the dorsal surface of the

rachis of an injected specimen to show the extrusion of carmine particles from four dorsal pores (*D.P.*); $\times 2$. These are the apertures of small transverse canals, which in this portion of the rachis establish communications dorsally between the large dorsal canal and the exterior. The particular specimen differed from any other which I have examined in the presence of a group of unusually large siphonozooids (*St.*) in the region superior to that of the dorsal pores. Elsewhere the siphonozooids (*St.*) are quite normal in character.

Fig. 6.—*Pennatulula phosphorea*. Drawing of the dorsal surface of the upper portion of the rachis of a young colony, $\times 15$, showing the terminal zooid and four dorsal pores (*D.P.*) from which, after injection, as in fig 1, carmine particles were extruded.

Fig. 7.—*Pennatulula rubra*. A portion of the dorsal surface of an injected specimen to show the extrusion of carmine particles from the mouths of the siphonozooids twenty-four hours after the injection was made. $\times 30$.

Fig. 8.—*Pennatulula rubra*. Transverse section through the stalk near to the base; $\times 15$. On the left side two of the smaller apertures are shown (*Ap.*) which communicate by means of the spaces in the spongy tissue with the ventral canal (*V.C.*). In the upper portion of the section a small canal (*C.*) is shown in the dorso-ventral septum (*D.V.S.*), which establishes communication between the lumen of the dorsal and ventral canals. Small canals (*Ca.*) are also shown leading from the large canals into the spaces of the spongy tissue (*Sp.T.*). A very thorough and complete system of communication is established by means of similar canals throughout the colony, which is thus brought into communication with the external sea-water. The epithelial tissue lining the canals is much vacuolated (Fig. 9) and contains numerous deeply staining cells, which are probably mucus-secreting, and are similar in character to cells composing the greater portion of the periphery of the base of the stalk. Immediately below this layer of vacuolated and deeply staining tissue, parallel with the periphery and outlining the large dorsal and ventral canals, is an extremely delicate sheath of transverse muscular fibres (*T.M.F.*), which becomes, however, much more pronounced in the superior regions of the stalk. The spongy tissue occupies the whole of the space between the canals and the body-wall.

Fig. 9.—*Pennatulula rubra*. A portion of the vacuolated and deeply staining epithelial tissue which lines the dorsal and ventral canals as seen in a transverse section of the stalk; $\times 800$. A similar epithelial tissue covers externally the basal portion of the stalk. The endoderm in this portion of the canal is curiously papillate in form, and extremely vacuolated. It contains numerous deeply staining cells, which probably have for their function the secretion of mucus, and also send off into a supporting mesogloëal core numerous muscle-fibres

(*M.F.*), which probably play an important part in the expansion and contraction of the canals. Many of the endodermal cells also contain ingested particles of the injected carmine, which have been conveyed to this portion of the colony by means of the circulating currents. The presence of carmine in the cells may indicate their possession of nutritive and excretory functions. The mucous secretion from the cells, which is no doubt responsible for the viscid character of the contents of the canals, may be lubricating in function, and may from its hygroscopic nature play an important part in the dilatation and contraction of the colony.

PLATE 27.

Figs. 10-15.

Fig. 10.—*Anthoptilum grandiflorum*. Drawing of the ventral surface of the stalk to show the folded thickened zone (*T.Z.*), principally due in this instance to an extraordinary growth of the spongy (distensible) tissue composing the internal body-wall, and not to the special development of an internal sphincter muscle as in *Pterœides* (fig. 14, *Sph.M.*). Both modifications of structure doubtless serve the same function in bringing about the dilatations and contractions of the colony. The lower portion of the thickened zone and the region immediately inferior to it is studded with numerous minute siphonozoids (*Si.*), which play an important part in maintaining the hydrostatic equilibrium of the colony. (Natural size.)

Fig. 11.—*Anthoptilum grandiflorum*. Dissection of the stalk from the ventral surface (two thirds natural size), showing the hook-like termination of the axis induced by the contraction of the muscular apparatus controlling it (*M.A.A.*), the terminal basal aperture of the dorsal canal (*B.D.Ap.*), and the thickened zone (*T.Z.*) in the upper portion of the stalk—composed of hydrostatic and muscular spongy tissue, which is produced into laminate processes (*L.M.P.*) presenting a free edge to the body-cavity. The outer surface of the thickened zone was observed, on microscopical examination, to be studded with minute siphonozoids of the normal Pennatulid type (fig. 10). This extraordinary development of the laminate processes appears to be unique and has not been observed in any other genus.

Fig. 12.—*Virgularia juncea*. Dissection of the stalk from the dorsal surface exposing the lumen of the dorsal canal (three quarters natural size). The stalk in this genus is characterised by its unusual length, tenuity, brittleness and extreme powers of contractility. In elemental constitution it is similar to *Pennatula* (fig. 13) differing only from that genus in proportionate development. As in *Pennatula* the muscular body-wall becomes attenuated towards the base, where it

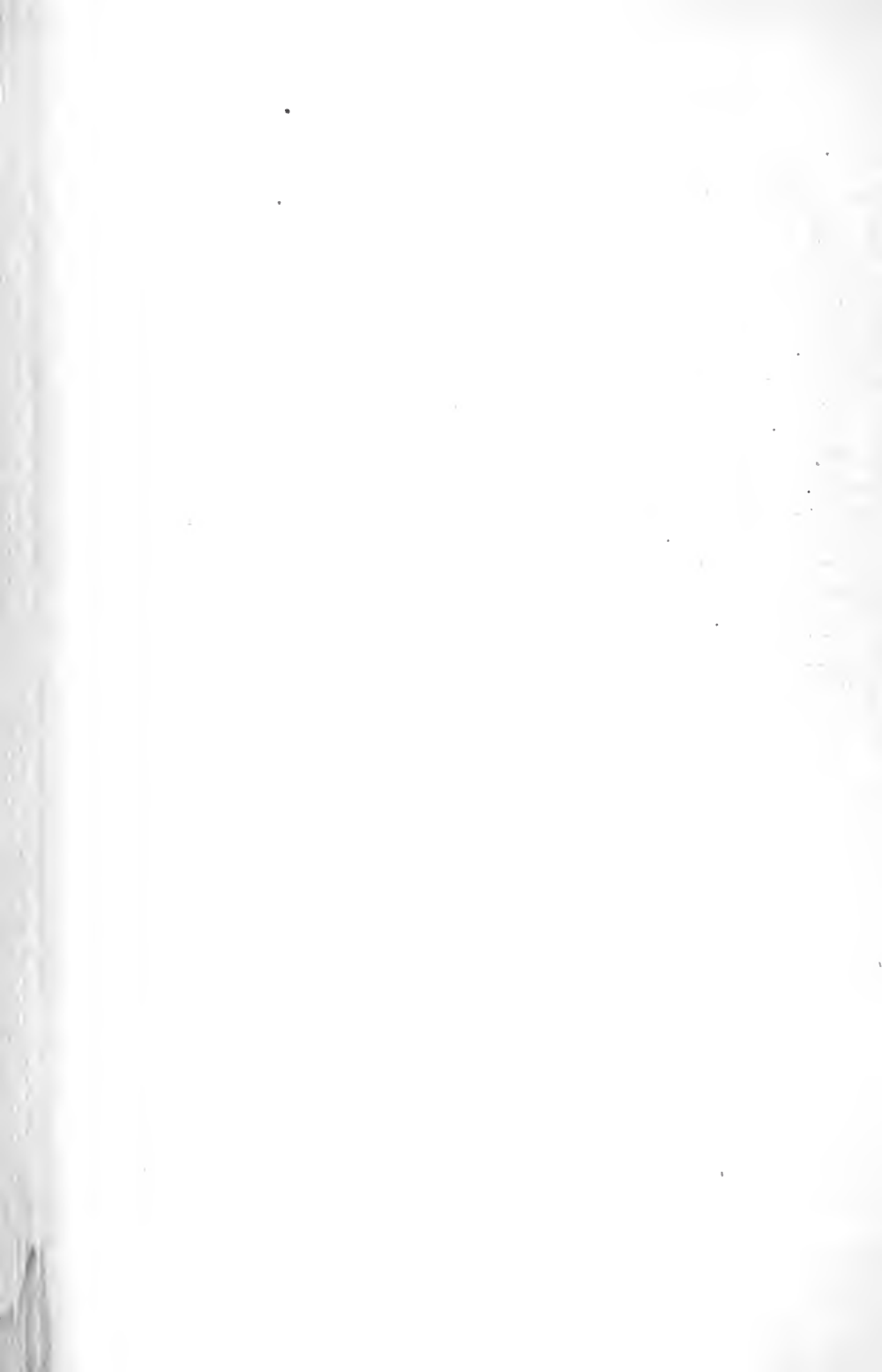
becomes delicate and membranous in character. In the specimen represented in the drawing the slender, needle-like, but slightly flexible axis (*Ax.*), supported by the axial sheath (*Ax. S.*), does not extend down to the base of the stalk as is usually the case in this genus (Marshall, p. 56). A close similarity exists between *Pennatula* and *Virgularia* in the musculature (*M.A.A.*) controlling the movements of the axis.

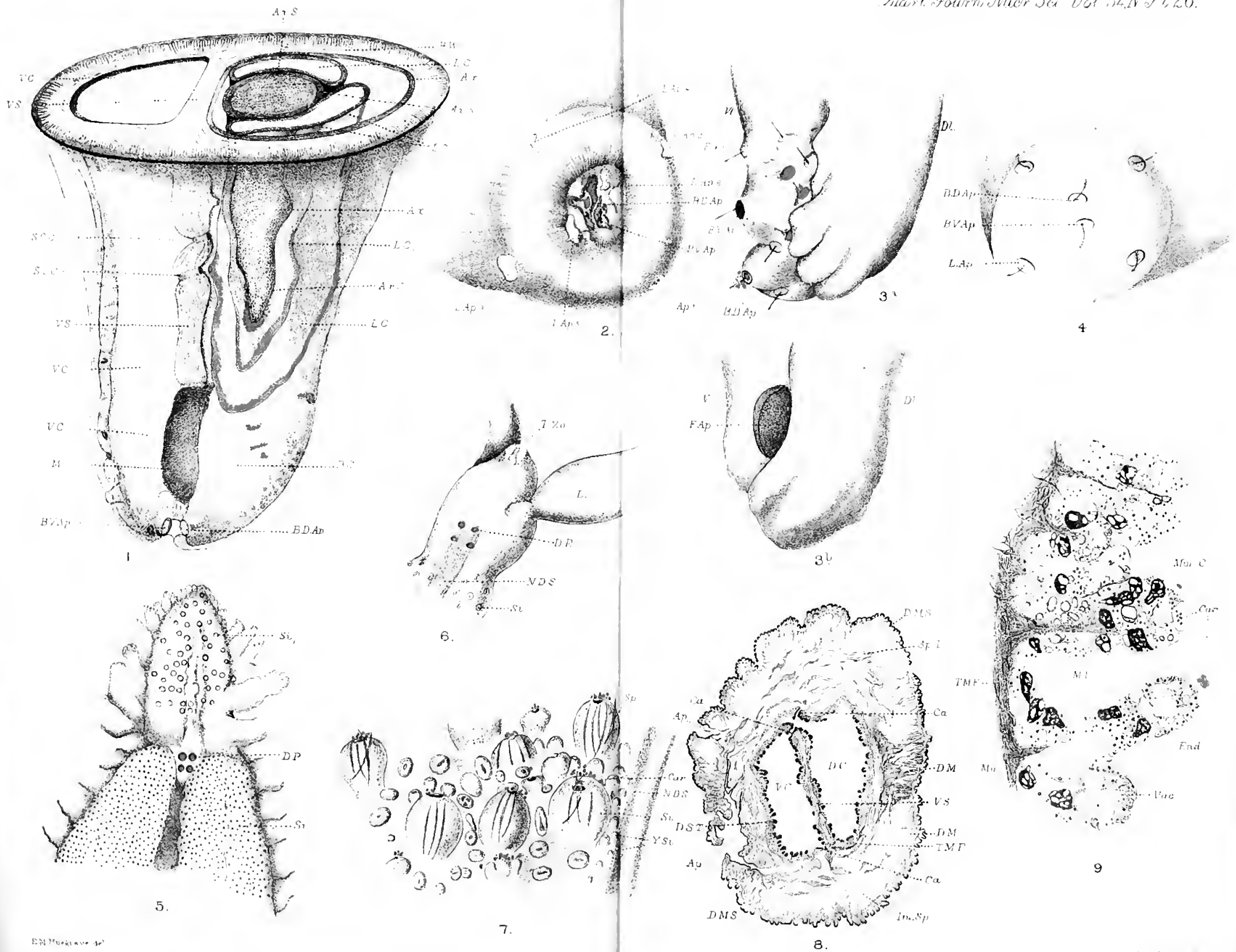
Fig. 13.—*Pennatula rubra*. Dissection of the stalk from the dorsal surface exposing the lumen of the dorsal canal; $\times 2$. The stalk is shorter and of comparative greater thickness than in *Virgularia* (fig. 12), but it is very similar in constitution. In *Pennatula* the four large canals of the stalk are shorter and broader, and the musculature controlling the axis less strongly developed. *Pennatula* appears to be less sensitive to contact, and therefore less contractile than *Virgularia*. In the drawing the vertical septum is seen to extend to the extreme base of the stalk, so that the ventral canal is equal in length in this region to the dorsal canal (compare *Pterœides* [fig. 14], *Anthoptilum* [fig. 10], etc.). The transverse muscular tissue (*T.M.F.*) of the body-wall in the neighbourhood of the insertion of the musculature controlling the axis is strongly developed to give the necessary additional support. The thickening of muscular tissue in this region is more strongly marked in *P. naresi* and *P. borealis*. In this respect *Pennatula* approaches *Pterœides*.

Fig. 14.—*Pterœides griseum* var. *longispinosum*. Dissection of the stalk from the dorsal surface exposing the lumen of the dorsal canal; $\times 1\frac{1}{3}$. The musculature of the body-wall is very pronounced in this genus. The thickened zone in the upper portion of the stalk is due in this genus to an extraordinary development of a sphincter muscle (*Sph.M.*), in which respect it apparently differs from all other genera. The fibres of the sphincter run almost transversely, and are attached on the inner side to the axial sheath and on the outer side to the body-wall. This powerful muscle has probably a double function—it may assist in the support of the axis, but its chief function seems to be that of controlling the dilatations and contractions of the stalk, and therefore materially assists in regulating the quantity of fluids within the canals. At the base of the sphincter muscle are powerful muscle-bands (*M.B.*), which are connected with the axial sheath and body-wall, and in addition assist in supporting and controlling the extremely powerful muscular apparatus (*M.A.A.*) governing the action of the axis (*Ax.*). The calcareous axis with its accompanying muscular sheath is slightly twisted spirally at its apical and basal termination (fig. 15). The whole conformation of the stalk seems to indicate that it is specially adapted as a boring organ, working, in this instance, in a screw-like fashion, and also as a pump working with a slightly piston-

like movement in the cavity of the dorsal canal when the musculature contracts. This would give an impetus in an upward direction to the currents entering at the basal apertures. As in other genera the musculature of the body-wall is less well developed near the base, its place being occupied by the spongy hydrostatic tissue, which is abundantly supplied with canals having numerous apertures into the lumen of the canals and also to the exterior (fig. 3). In living specimens this portion of the stalk is often considerably dilated. In the drawing numerous apertures (*Ap.*) are indicated, which are arranged in two vertical rows, one on the left the other on the right of the drawing, between the insertion of the muscular fibres of the muscular apparatus controlling the axis, which establish communications between the lumen of the large canals and the canal of the spongy tissue lining the body-wall.

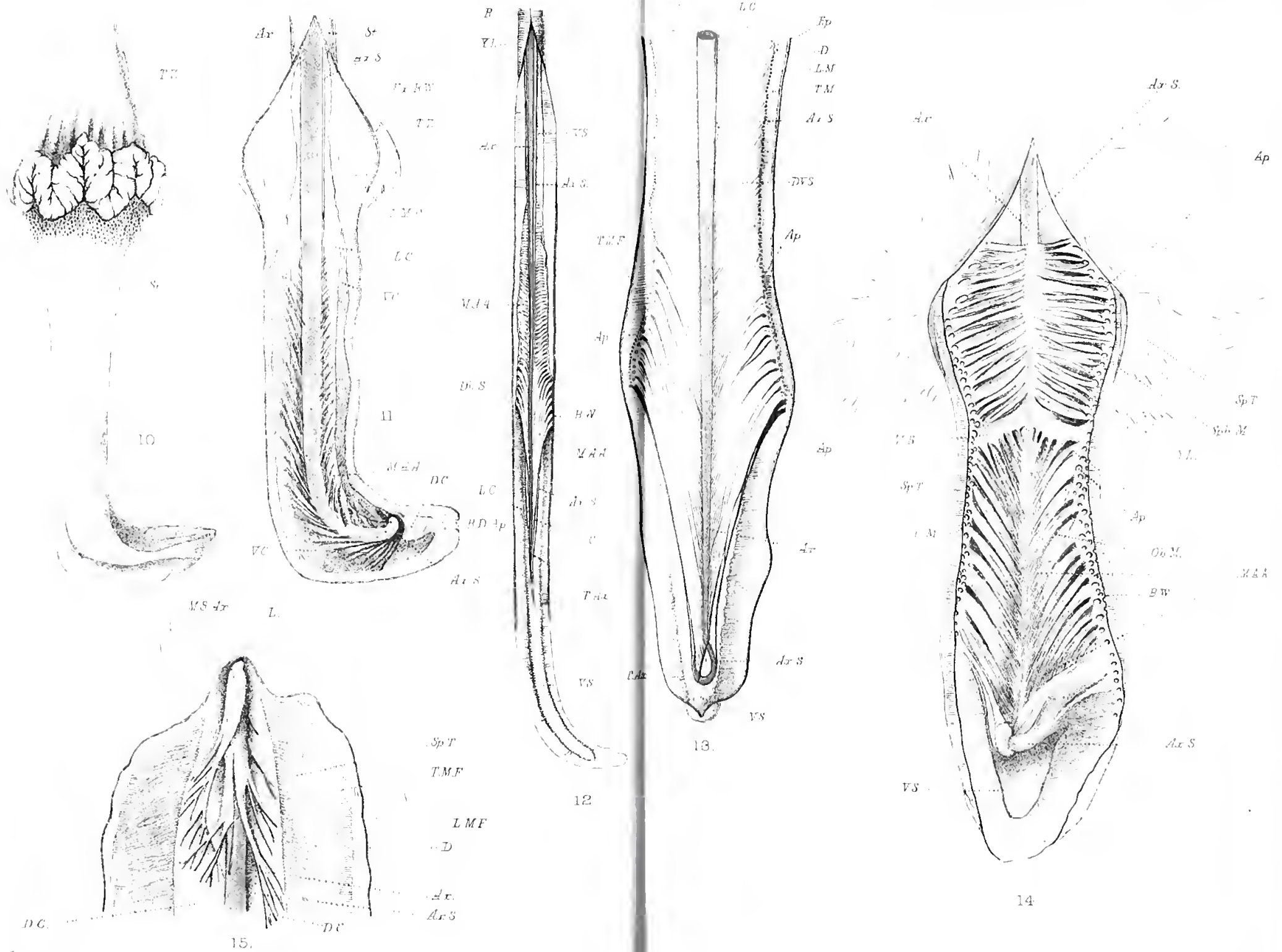
Fig. 15.—*Pterœides caledonicum*. Dissection of the upper portion of the rachis from the dorsal surface exposing the lumen of the dorsal canal (*D.C.*) to show the musculature controlling the apical portion of the axis, which is slightly twisted spirally when the muscles are contracted, as indicated in the drawing; $\times 1\frac{1}{3}$. The corresponding twist of the basal portion of the axis in this genus is indicated in fig. 14. The musculature and thickness of the body-wall is more pronounced in this specimen than in that of the species *Pt. griseum* (fig. 14).













On Certain Features in the Development of the Alimentary Canal in *Lepidosiren* and *Protopterus*.

By

J. Graham Kerr,

Professor in the University of Glasgow.

With 13 Text-figures.

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I. INTRODUCTION.

THE following pages contain a short description of certain features which have seemed of special interest in the development of the alimentary canal of *Lepidosiren* and *Protopterus*. The technical methods used have been the same as those used in earlier stages of my work. In particular, I have made constant use of the method of reconstruction by means of ground glass plates, and I have endeavoured to use both the celloidin and the paraffin methods of embedding. As I have already had occasion to point out more than once, it is, in my opinion, essential in embryological investigations to use these methods side by side. Both are liable to mislead, both have their faults, but the faults are different in the two

cases, and by a careful and critical use of the two, error can be to a great extent eliminated.

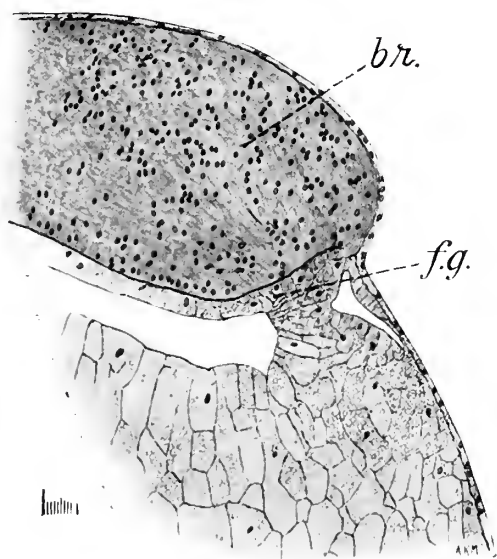
I have, as on other occasions, to acknowledge the valuable assistance which I have received from Mr. Maxwell, to whom I owe the drawings illustrating this paper, and to Mr. P. Jamieson, who has done the necessary section cutting.

II. DIFFERENTIATION OF THE MAIN REGIONS OF THE ALIMENTARY CANAL.

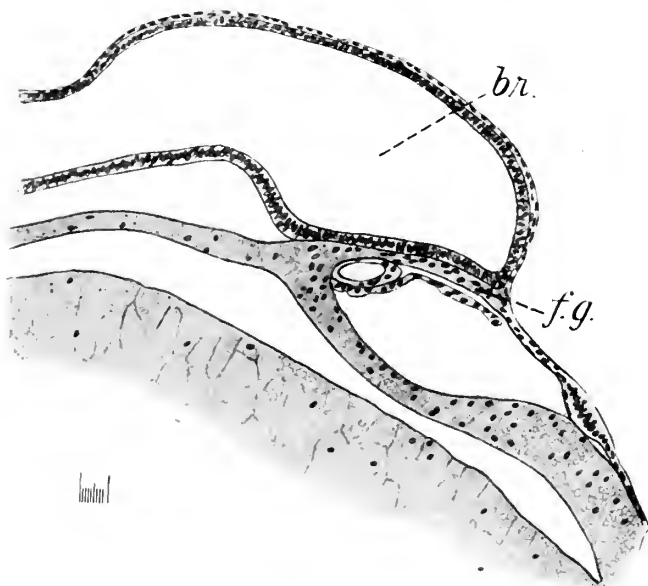
The alimentary canal of the adult *Protopterus* or *Lepidosiren* becomes developed out of the mass of primitive endoderm, characterised by its large cells and by the large size of the yolk granules with which their protoplasm is laden. The almost spherical mass of endoderm of the early embryo becomes fashioned into the tubular alimentary canal of the adult by a complicated process of what I have somewhat loosely termed modelling, the general course of which is illustrated by the figures in this paper. It is necessary to state quite definitely that, in using the term modelling, I do not for a moment mean to suggest that this process is carried out by the active agency of the surrounding tissues upon a plastic and passive endoderm. On the contrary, I believe it to be essential, in all embryological work, to bear constantly in mind that the organ is only a part of the organism; that any organ or piece of tissue is throughout its development in intimate physiological connection with its surrounding tissues, and that to consider it by itself, without reference to these surrounding structures, is to make use of a method which is almost certain to lead to grave error. The alimentary canal, then, of the developing *Lepidosiren* or *Protopterus* is, during its process of "modelling," by no means to be assumed to be passive; the whole process is one of co-operative activity between the endodermal rudiment and the mesodermal structures in relation with it.

The first stage of the modelling of the alimentary canal is that in which a narrow anterior part—the fore-gut—becomes

TEXT-FIG. 1 A.



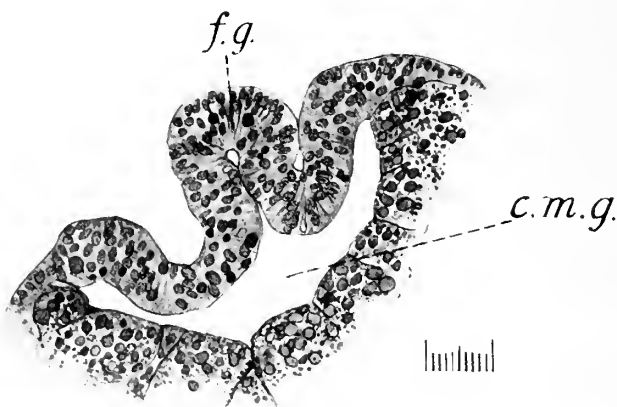
TEXT-FIG. 1 B.



TEXT-FIGS. 1 A, B.—Sagittal sections illustrating the folding off of the fore-gut. A. *Protopterus*, stage XXIII. B. *Lepidosiren*, stage XXV. *br.* Brain. *f.g.* Fore-gut.

differentiated from the larger hinder region (mid-gut and hind-gut) which forms the main storehouse for the yolk. The commencing development of the fore-gut is illustrated by text-fig. 1 A, in which the fore-gut rudiment is beginning to be nipped off from the rest of the endoderm by the development of a chink beneath it. This chink then gradually spreads backwards, as shown in text-fig. 1 B, and also laterally, and in this way the fore-gut becomes demarcated, the

TEXT-FIG. 2.



Section through junction of fore- and mid-gut of a *Lepidosiren* larva of stage XXXIV, showing the origin of the pyloric valve.
c. m. g. Cavity of mid-gut. *f. g.* Hind end of fore-gut.

ventral part of the space alluded to becoming occupied by the pericardiac cavity with its mesodermal lining. The folding off of the fore-gut is continued backwards until, about stage XXXII, it reaches the level of the pylorus, after which it increases in length by its own growth, and undergoes gradual histological differentiation until the adult condition is reached. About stage XXXIV the active growth in length of the fore-gut causes it to push its hinder end into the cavity of the mid-gut, the wall of which is relatively thin at the point of junction of fore- and mid-gut (see text-fig. 2). The flattened spout-like projection of the hinder end of the fore-

gut into the cavity of the mid-gut persists throughout life, and forms the characteristic "pyloric valve."

The main features in the topographical evolution of the mid-gut and hind-gut will be gathered from an inspection of the figures of the external features (see Keibel's "Normentafeln," Heft x) together with text-figs. 3 A—D, 4 A—D, and 5.

In correlation with the function of this part of the gut as the storehouse of food material on which the young animal has to subsist for a prolonged period, its cells remain for long laden with yolk, and its developmental progress is correspondingly retarded. The first conspicuous change consists in the rapid elongation of the hinder part of the gut which accompanies the rapid growth of the hinder trunk region (see figures of external features, stages XXV—XXX). The anterior region of the mid-gut for a considerable time retains its spheroidal shape, and it is this which gives the characteristic tadpole-like appearance to these stages. About stage XXXIII in *Lepidosiren*, but not till about stage XXXV in *Protopterus*, the prominent bulging of the anterior part of the mid-gut disappears, and the tadpole-like appearance is finally lost.

The general appearance of the mid- and hind-gut as seen in a dissection of a young *Lepidosiren* of stage XXXII is shown in text-figs 3 A and 4 A. The prominent rounded bulging of the anterior end is already at this stage giving place to a gradual tapering. Towards the hinder end a faint spiral marking caused by a shallow groove traversing the surface of the gut rudiment foreshadows the development of the spiral valve.

In the mid-dorsal line a broad valley passes back for some distance, and in this lies the rudiment of the lungs. Text-fig. 4 A shows how the fore-gut in the region of the glottis bends abruptly towards the left side, passing into the mid-gut far to the left of the mesial plane.

In the dissection of stage XXXV (text-figs. 3 B, 4 B and 5) it is seen that the swollen mass of yolk in front has been greatly

reduced, and this part of the gut no longer bulges conspicuously. The spiral valve is now indicated by a deep

TEXT-FIG. 3 A.



TEXT-FIG. 3 B.



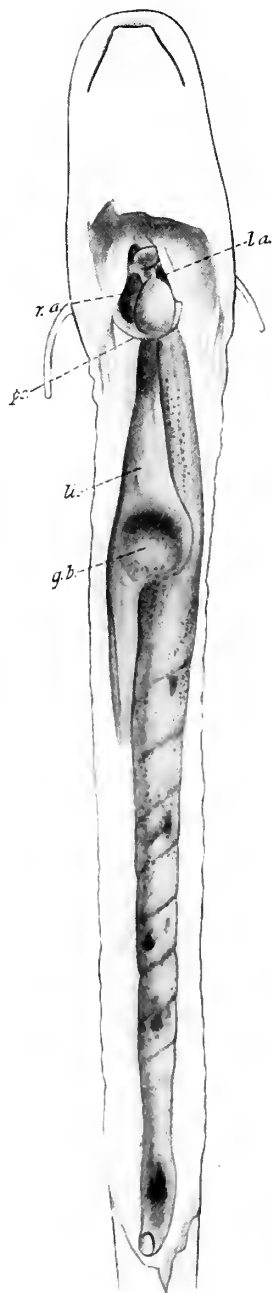
TEXT-FIGS. 3 A, B, C, D.—Dissection of young *Lepidosirens* of stages XXXII, XXXV, XXXVI, and XXXVII, from the ventral side, illustrating the development of the alimentary canal. *g. b.* Gall bladder. *ht.* Heart. *l. a.* Left auricle. *li.* Liver. *pc.* Pericardium. *r. a.* Right auricle.

“incision,” which traverses the mid-gut right to its anterior end, but is absent for a short distance posteriorly.

TEXT-FIG. 3 C.



TEXT-FIG. 3 D.



At stage XXXVI (text-figs. 3 c and 4 c) the intestinal rudiment forms a spirally coiled structure, the turns of the

TEXT-FIG. 4 A.

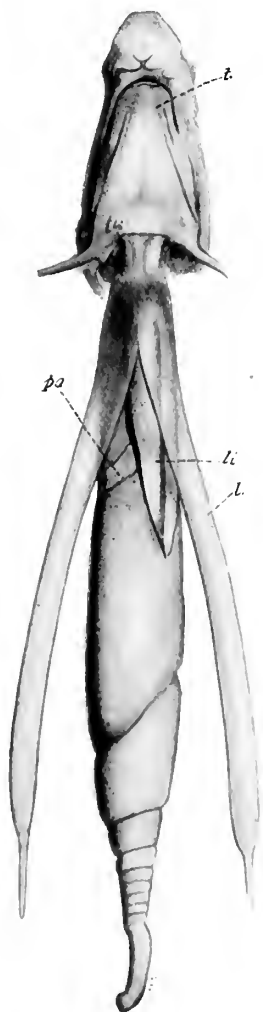
TEXT-FIG. 4 B.



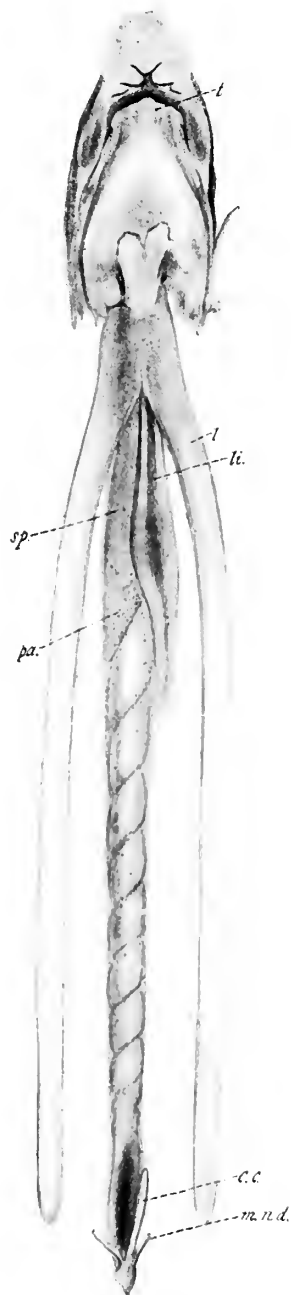
TEXT-FIGS. 4 A, B, C, D.—Dissections of the mid-gut of young *Lepidosirens* seen from the dorsal side. A. Stage XXXII. B. Stage XXXV. C. Stage XXXVI. D. Stage XXXVII. c.c. Cloacal caecum. f.g. Fore-gut. l. Lung. li. Liver. m.n.d. Mesonephric duct. pa. Pancreas. pa.d. Dorsal pancreas. ph. Pharynx. sp. Spleen. t. Tongue.

spiral being separated by the deep incision mentioned in the preceding stage. In all probability this spirally coiled condi-

TEXT-FIG. 4 C.



TEXT-FIG. 4 D.



tion of the intestine may be looked on as a repetition of a phylogenetic stage,¹ in which the intestine had assumed a spiral coiling owing to its relatively great length in comparison with the length of the splanchnocœle. As was shown especially by Rückert² the spiral valve in certain Elasmobranchs is similarly preceded by a spirally coiled condition of the endodermal gut rudiment, in this case definitely associated with actual growth in its length. As, further, the spiral valve is characteristic of all the more primitive groups of fish-like Gnathostomata, Elasmobranchs, Crossopterygians, and Lung-fishes, not to mention Actinopterygians and ancient groups of amphibians and reptiles, we may take it as fairly probable that the Gnathostomata were as a whole characterised during an early period of their evolution, a period antecedent to the splitting up into the groups above named, by the possession of a long spirally coiled gut.³

In comparing the stage under discussion (XXXVI) with the preceding stages it will be seen that the using up of the yolk is still taking place most actively in the anterior region. As a consequence it is seen that the first turn of the spiral has become greatly reduced in size, so that, as shown in ventral view, it is decidedly smaller than the succeeding turn instead of being much larger, as was the case in stage XXXV. Now that the turns of the spiral are distinct it may be seen that there are in all most usually nine or ten turns.

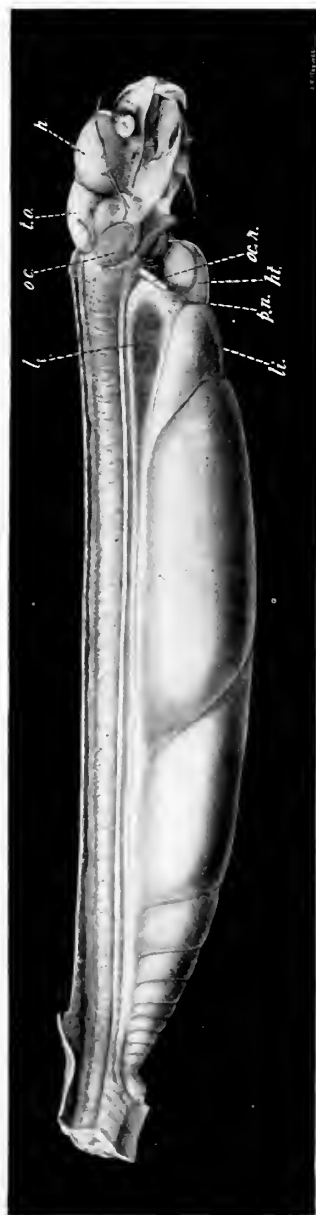
In the last stage figured (stage XXXVII, text-figs. 3 D and 4 D) the yolk has been used up to such an extent as no longer to influence the outward form of the intestine. The turns of the spiral are now of approximately uniform diameter, they are closely bound together by mesenchyme, and are enclosed

¹ Graham Kerr, 'Phil. Trans. Roy. Soc.' B. excii, 1900, p. 325.

² 'Arch. f. Entwickl. Mech.' iv, 1896, p. 298.

³ In all probability the gut has varied much in length from time to time during the evolution of the vertebrata, changes in length being associated with changes in the nature of the diet, e.g. from vegetable to animal.

TEXT-FIG. 5.



Dissection of a young *Lepidosiren* of stage XXV from the right side. *h*, Hemisphere, *ht.*, Heart, *l*, Lung, *lt.*, Liver, *o.c.*, Auditory capsule, *oc.r.*, Occipital rib, *p.n.*, Pronephros, *t.o.*, Tectum opticum.

in a cylindrical sheath of splanchnic mesoderm dotted with scattered chromatophores. From being a spirally coiled structure the intestine has therefore now assumed the outward form of a straight cylinder, only the cavity in its interior betraying its once coiled condition.

III. BUCCAL CAVITY.

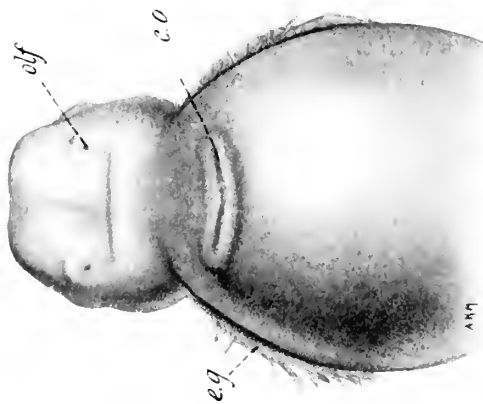
The Dipnoi share with various Amphibians the peculiarity that the main part of the buccal cavity arises in place of the anterior portion of the yolk-laden enteric rudiment derived from the macromeres of the segmented egg, i.e. its wall is in great part derived from a mass of cells which would ordinarily be called endoderm. Those who are sticklers for the sanctity of the germ layer theory find it difficult to accept this statement, and would rather believe that, although the definitive buccal cavity comes into existence in place of a "primitive endoderm" structure, yet its lining is formed by a definite ingrowth of ectoderm. Thus the buccal cavity of the forms mentioned would be not merely in theory, but as regards its actual ontogenetic development a typical stomodæum. GREIL in particular, who has investigated the development in *Ceratodus*, appears to have no doubt that actual ingrowth of cells from the outside takes place to form the lining of the buccal cavity. Personally I see no reason to depart from the statement which I made some years ago¹ that the main part of the buccal lining arises in situ by actual transformation of the originally yolk-laden cells. The active metabolism associated with this process of transformation is here as elsewhere accompanied by a breaking up of the yolk granules into very fine particles so that they may be more easily assimilable, and it is this assumption of a finely yolked and richly protoplasmic character that causes the cells to assume an ectoderm-like appearance. "It is," as I put it in my former paper, "as if an influence were spreading inwards

¹ 'Quarterly Journal of Microscopical Science,' vol. 46, p. 423.

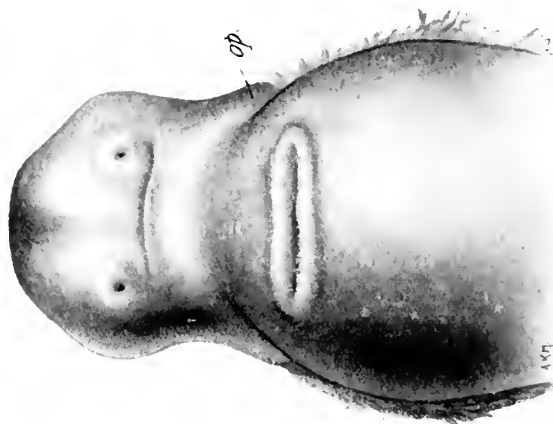
from the external epiblast, gradually transforming the original "endoderm" yolk-laden cells into ectoderm like itself." Apart from the difficulty of believing in the possibility of a layer of soft protoplasmic ectoderm cells growing inwards and pushing aside compact masses of yolk-granules without even producing any signs of mechanical disturbance of the tissues, the study of carefully prepared celloidin sections is, I think, sufficient to convince anyone that it is really a process of conversion *in situ* which is taking place. In such sections there is frequently visible a quite broad zone of transition in which the richly protoplasmic "ectoderm" cells pass by imperceptible gradations into the typical yolk-cells, there being no trace of the absolutely sharp boundary which must be present were the ingrowth hypothesis correct.

To the main part of the buccal cavity, which arises by cytolysis in the midst of an originally solid mass of yolk-cells, and the walls of which give rise to the teeth, as has been described in Part III, there becomes added in later stages of development the antero-lateral part of the definitive buccal cavity, in the roof of which are situated the narial openings. This additional part of the buccal cavity arises in ontogeny in the same kind of way as the whole cavity does in *Polypterus*, i.e. by the walling in of a space on the lower side of the head, through the development of the upper lip and the forward growth of the lower jaw. The mode of development of this part of the buccal cavity is made clear by text-fig. 6 A—F. In stages XXXI and XXXII of *Protopterus* (text-fig. 6 A and B) the position of the front end of the alimentary canal is marked out in a ventral view of the larva by a transverse line—the line of junction of the yolk-laden enteric cells with the ectoderm. Some little distance in front of the outer end of this line upon each side is seen a dimple, which marks the olfactory rudiment. In a specimen rather younger than stage XXXIV in its general features the olfactory dimple (text-fig. 6 c) is seen to have become elongated in an oblique direction, so that its long axis passes from

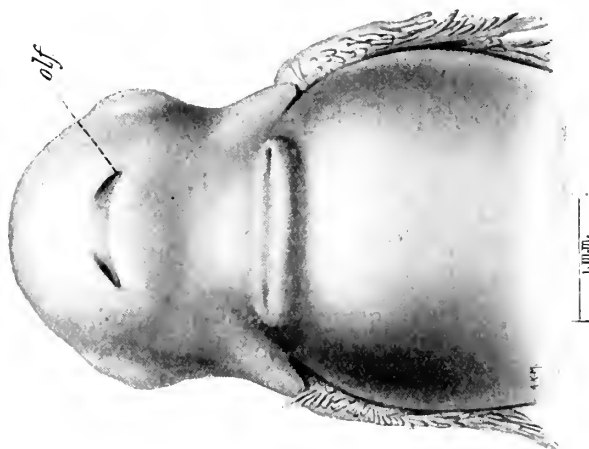
TEXT-FIG. 6 A.



TEXT-FIG. 6 B.



TEXT-FIG. 6 C.

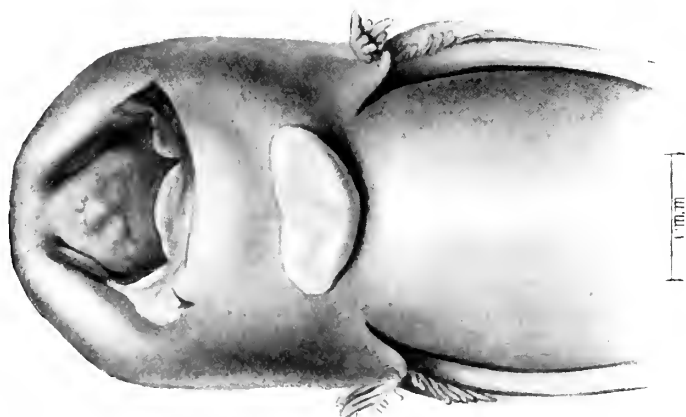


TEXT-FIGS. 6 A, B, C, D, E, F.—Views of under-surface of head in Protopterus of stages XXXI, XXXII, XXXIV—, XXXV, XXXV and XXXVI—, to show the relations of the olfactory organs. In E and F the floor of the buccal cavity has been partially cut away. *e. g.* External gill. *olf.* Olfactory rudiment. *olf. a.* and *olf. p.* Anterior and posterior nares. *op.* Operculum. *to.* Tooth.

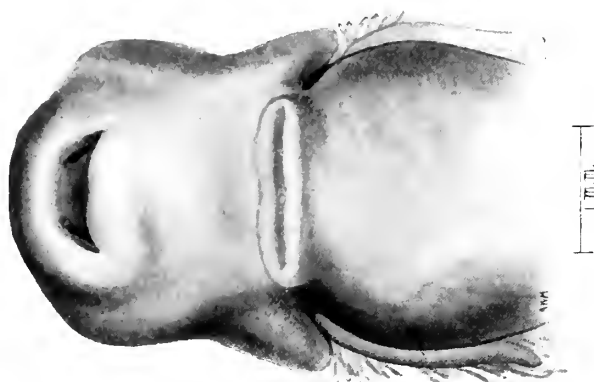
TEXT-FIG. 6 F.



TEXT-FIG. 6 E.



TEXT-FIG. 6 D.



in front backwards and outwards. It now forms a deep cleft leading right into the interior of the olfactory organ along nearly its whole length. (The internal cavity of the olfactory organ is at first closed, and arises in the midst of the originally solid rudiment, as explained in Part III.)¹

The area containing the two olfactory clefts is now marked off from the rest of the under surface of the head, behind by a sharp fold—the first indication of the lower lip, and in front by the much less sharply marked rudiment of the upper lip. The area within these folds, and having an olfactory cleft upon each side, is the rudiment of that additional antero-lateral part of the buccal roof which becomes added on to the posterior and larger portion derived from the solid mass of yolk-cells.

By stage XXXIV (text-fig. 6 D) the delimitation of this additional part of the buccal roof from the rest of the under surface of the head has become more sharply marked, the upper lip being now more prominent, and the lower lip or lower jaw having commenced to grow forwards to form its floor. The olfactory cleft is more elongated. It has become drawn out and narrowed in its middle part to a fine slit, which connects the dilated anterior and posterior ends—the rudiments of the anterior and posterior nares. Of these the posterior naris is now hidden in a ventral view of the head, owing to the forward growth of the lower jaw.

In stage XXXV it is necessary to cut away part of the lower jaw to see the olfactory clefts. It is seen (text-fig. 6 E) that the lips of the cleft are now in close apposition except at their ends, and between this stage and stage XXXVI the lips undergo complete fusion, so that the anterior and posterior nares are now distinct openings (text-fig. 6 F).

Keibel has remarked:² “The so-called upper lip of the *Dipnoi* lies morphologically further outwards than does the mouth margin in any other Vertebrates.” This character is specially expressed in the fact that the margin of the mouth

¹ ‘Quarterly Journal of Microscopical Science,’ vol. 46, p. 438.

² ‘Anat. Anz.,’ vol. viii, p. 487.

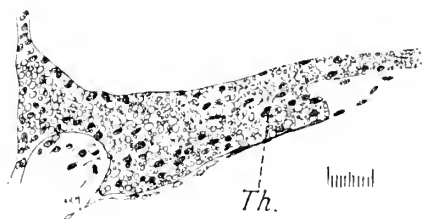
encloses anterior as well as posterior nares, and forms one of the most distinctive features of the Dipnoi. How this arrangement has come about in Phylogeny does not seem quite certain, but most probably it has been by a backward migration of the narial rudiment, rather than by an extension forwards of the mouth boundary. It will be noticed that the upper lip, while very prominent laterally, can hardly be said to exist in the region near the mesial plane. This gives a characteristic gaping, almost cyclostomatous, appearance to the mouth of the young Dipnoan,¹ and if it be assumed that this is a repetition of a phylogenetic condition, it is clear that a backward migration of the narial openings into the buccal cavity could readily have taken place. The physiological significance of the intrabuccal position of the narial openings is clearly in all probability adaptive to the mud-burrowing habits. The olfactory organ is in the living Dipnoan used, so far as my observations go, entirely as a sense organ, its respiratory function not yet having developed. The sense of "smell" affords the principal means by which the living *Lepidosiren* or *Protopterus* finds its food; a little colouring matter, e.g. blood, in the water, shows how they actively "sniff" about, with snout sharply bent down, in search of food particles at the bottom of the water.

Glands and Sense Organs.—Unicellular glands and sense buds are scattered, as already shown by Parker for *Protopterus*, over the lining of mouth and pharynx as on the outer skin, while the flask-shaped glands so characteristic of the external ectoderm are normally absent from the buccal cavity.

Thyroid.—The thyroid makes its appearance about stage XXX in *Lepidosiren* as a solid keel-like projection from the ventral side of the solid buccopharyngeal rudiment. The study of sagittal sections (text-fig. 7) show that the thyroid rudiment becomes gradually cut off from the buccopharyngeal mass from behind.

¹ Even in the adult *Lepidosiren* the mouth is actively suctional, and food is drawn into the mouth by a strong sucking action.

TEXT-FIG. 7 A.



TEXT-FIG. 7 B.



TEXT-FIG. 7 C.



TEXT-FIG. 7 D.



TEXT-FIGS. 7 A, B, C, D.—Sagittal sections showing origin of thyroid in *Lepidosiren*. A, B, and C. Stage XXX. D. Stage XXXI. *th.* Thyroid. *t.* Tongue.

About stage XXXI (*Lepidosiren*) the narrow isthmus which still unites the thyroid to the buccal floor in front of the tongue becomes severed, and the organ lies free as a rounded mass of coarsely yolked cells.

Between stages XXXIV and XXXV the thyroid rudiment becomes broken up into strands by intruding mesoderm with blood-vessels, and a little later the strands are broken up into typical rounded follicles with colloidal secretion in their interior.

Tongue.—Reference to the text-figures (7, A—D) illustrating the development of the thyroid is sufficient to show that the tongue of the *Dipneumona* is a primary tongue, like that of *Urodele* Amphibians, except that in this case no gland field develops in connection with the tongue, at least up to stage XXXVIII. The downgrowth from the solid buccal rudiment, from the posterior side of which the thyroid is developed, becomes split about stage XXXI, and it is this splitting which causes the portion of buccal floor behind it to be bounded in front and laterally by a deep cleft so as to form a distinct tongue.

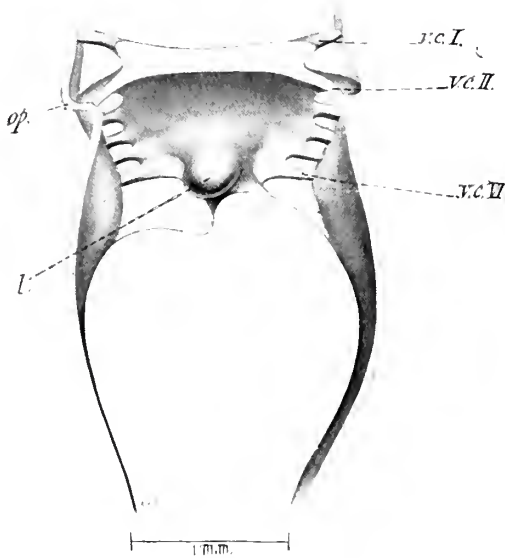
IV. LUNG.

The first rudiment of the lung is seen in text-fig. 8, A, which is a ventral view of the pharyngeal region of a *Protopterus* of stage XXXII.

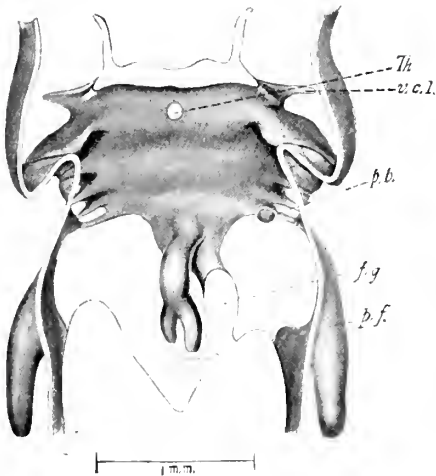
The lung rudiment is seen to form a rounded bulging from the pharynx in the mid-ventral line just at the level of cleft VI. Pharynx and lung rudiment are alike solid at this stage.

The endodermal lung rudiment grows at first ventralwards, and slightly towards the right side (text-figs. 8 and 12, A, pages 502, 514). Meanwhile the modelling of the œsophagus is proceeding; it becomes more elongated, more slender, and becomes displaced more and more towards the left side of the body. The lung rudiment soon begins to bend somewhat dorsally, and then continues to grow directly backwards. It

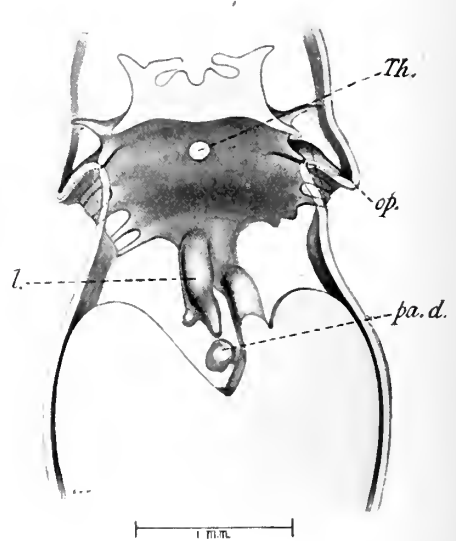
TEXT-FIG. 8 A.



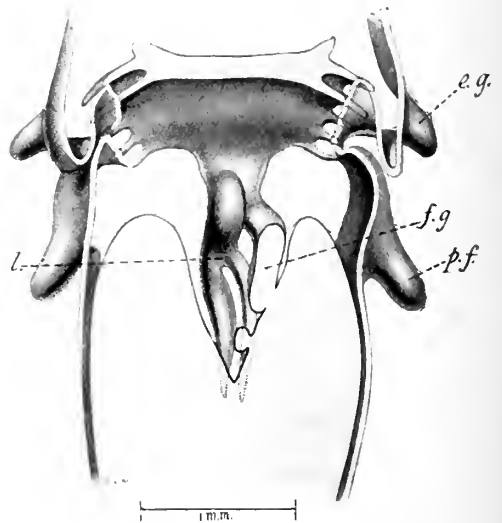
TEXT-FIG. 8 C.



TEXT-FIG. 8 B.



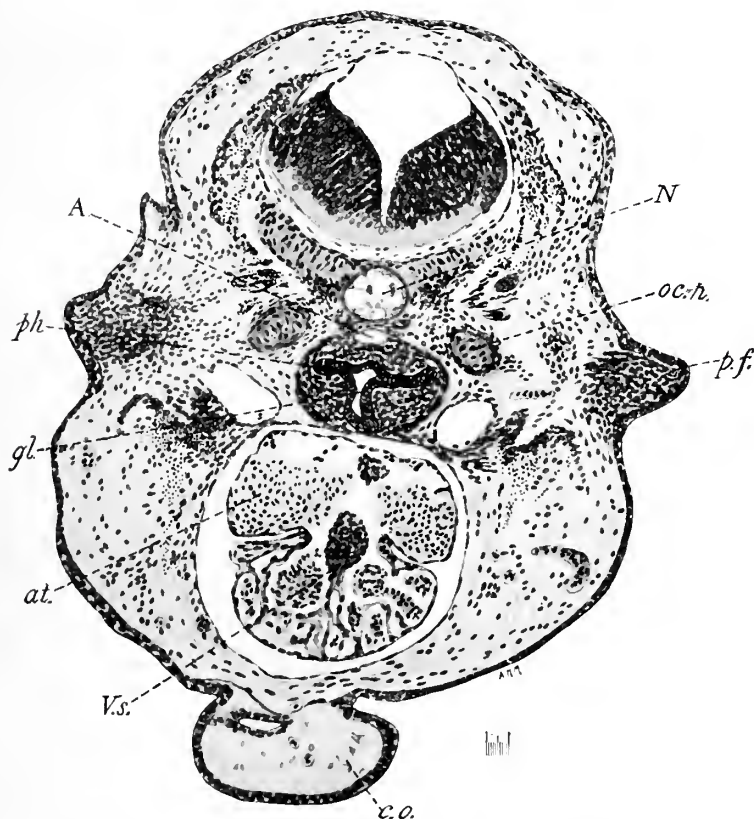
TEXT-FIG. 8 D.



TEXT-FIGS. 8 A, B, C, D.—Reconstruction seen from the ventral side of a thick horizontal slice through the pharyngeal region of *Protopterus*, illustrating the early development of the lung. A. Stage XXXII. B. Stage XXXIV. C. Stage XXXIV. D. Stage XXXV. *e. g.* External gill. *f. g.* Fore-gut. *l.* Lung. *op.* Operculum. *pa. d.* Dorsal pancreas. *p. b.* Post-branchial body. *p. f.* Pectoral limb. *th.* Thyroid. *v. c.* Visceral cleft rudiment. (Cut surfaces are indicated by the light tone.)

is enabled to do this by the already mentioned displacement of the œsophageal rudiment towards the left side. In specimens where the displacement of the œsophagus has not

TEXT-FIG. 9.



Transverse section of a young *Lepidosiren* of stage XXXIV through the region of the glottis. *A.* Aorta. *at.* Atrium. *c. o.* Cement organ. *gl.* Glottis. *N.* Notochord. *oc. r.* Occipital rib. *p. f.* Pectoral limb. *ph.* Pharynx. *v. s.* Ventricular septum.

taken place to so great an extent, the lung grows round it in the manner to be described later.

By stage XXXIV the hind end of the lung rudiment is distinctly bilobed, the right lobe being for some time relatively

small and inconspicuous as compared with the left (text-fig. 8, b and c).

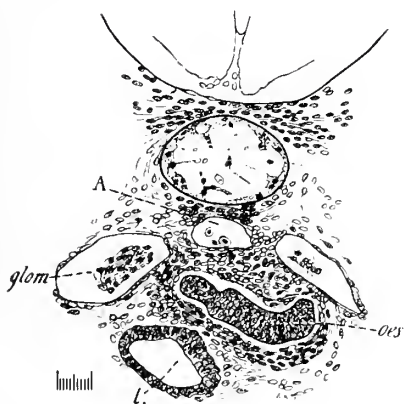
By stage XXXV (text-fig. 8, d), the two cusps of the lung rudiment are seen to be growing actively. They are now approximately of equal length, and they extend back for a short distance along the dorsal side of the main mass of yolk, their tips lying in the shallow valley upon its dorsal surface.

Subsequent stages in the development of the lungs will be made clear by text-figs. 4, A—D (pages 490, 491) representing dissections of young *Lepidosirens* of stages XXXII, XXXV, XXXVI, and XXXVII. It will be noticed how the lungs gradually extend backwards, at first in the shallow valley, already mentioned, on the dorsal side of the enteron. Later on they lie well above the surface of the enteron and, indeed, eventually, as will be shown later, dorsal to the entire splanchnocœle.

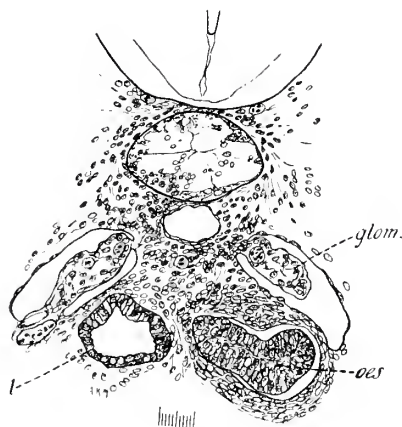
The lung rudiment is at first quite solid, but a cavity soon begins to develop in it, the pharynx at this level still remaining solid. By about stage XXXII in *Lepidosiren* the lung has become hollow throughout, although the pharynx at the level of the glottis is still solid. It is not till about stage XXXV that the lumen of the pharynx is completed, and there is an open glottis leading into the lung. It was at this same stage that the young *Lepidosirens* were observed first to swallow air, so we may take it that the lung is functional practically from the time at which its communication with the exterior is established. Correlated with this the mesodermal sheath of the lung is highly vascular by this stage (XXXV), and in places the blood-vessels are seen to penetrate the endodermal lining.

Torsion of the lung.—During the course of its development the lung undergoes a complicated process of torsion, which introduces considerable difficulties in the way of its investigation. Indeed, had not extensive material been available, it would in all probability have proved impossible to make out exactly what happens. The difficulty is due to the fact that torsion of the lung rudiment takes place successively in two opposite directions.

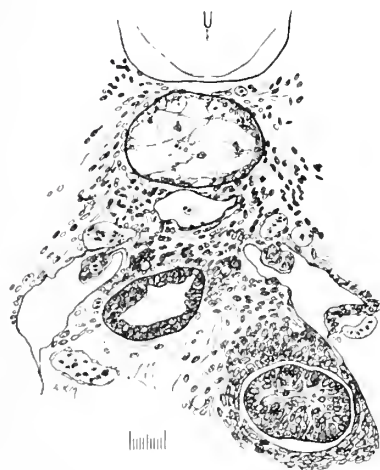
TEXT-FIG. 10 A.



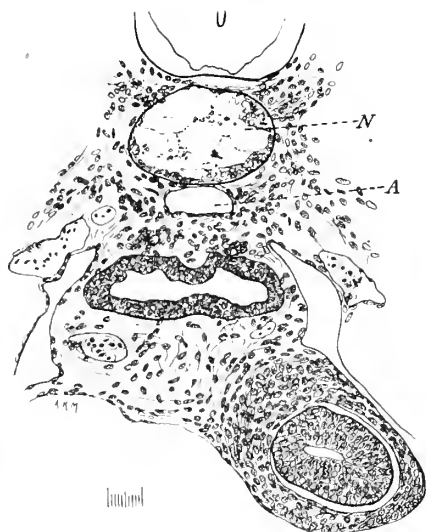
TEXT-FIG. 10 B.



TEXT-FIG. 10 C.



TEXT-FIG. 10 D.



TEXT-FIGS. 10 A, B, C, D.—Sections from the same series as that shown in text-fig 9 to illustrate the changing relations of lung to gut as it passes backwards. A, Aorta. *glom.* Glomus. *l.* Lung. *n.* Notochord. *es.* Esophagus.

There is first what may be called the primary torsion of the lung, which is illustrated by the camera drawing in text-figs. 9 and 10, A—D. During the early stages of its development the lung rudiment, while increasing in length, describes a spiral curve¹ round the œsophagus. Starting from the mid-ventral glottis it grows first ventralwards, tailwards, and towards the right side; then dorsalwards and tailwards; and, finally, tailwards and towards the mesial plane, until it attains its mid-dorsal position over the gut, after which it grows directly tailwards. Now, during the spiral part of this course, in addition to changing its position relative to the gut (being first ventral, then on the right, and finally dorsal), the lung undergoes what I have called the primary torsion—torsion about its own long axis in a counter-clockwise direction, as seen from the tailward direction. This torsion takes place through 180° , so as to cause a complete reversal in position of the hinder part of the lung rudiment at this stage, its morphologically ventral aspect becoming dorsal, its originally right side becoming left. The process of torsion is continued still further, however, in the majority of specimens, for it may be as much as 30° or 40° . The result is that in such specimens, when the tip of the lung begins to bifurcate, the (actual) left lung is seen to be considerably displaced towards the ventral side, as compared with the right (see text-figs. 8, B and C). This difference in level soon becomes corrected by processes of differential growth, in which a secondary torsion takes place, so that the two lungs are brought to the same level. It will be seen that this secondary torsion is in a direction the reverse of the primary torsion (i. e. it is clockwise, as seen from the tail end).² The

¹ Only visible in occasional individuals. More usually the œsophagus is sufficiently out of the way to the left side to render the curving no longer necessary.

² The primary torsion of the œsophagus is clearly of the type which would naturally be associated with a spiral coiling of a dextral type like that of the mid-gut, while the secondary torsion may express a tendency to return to the original condition. The possibility is

occurrence of these two torsional processes in opposite directions introduce, as will readily be understood, very puzzling and deceptive appearances into the sections of larvæ of such stages of development.

Topographical Relations of Lung to Cœlom.—In its earliest stages the endodermal lung rudiment is naturally enclosed within the mesenchymatous tissue of the splanchnopleure. In a *Protopterus* of stage XXXIV the root of the lung retains these relations. If, however, sections farther back towards the hind end of the lung rudiment are examined it is found that the œsophagus has, in this region, bent away towards the left side, and has become freed from the dorsal mesentery, which passes direct from the under surface of the dorsal aorta to the upper surface of the liver. The lungs grow directly backwards in the substance of this dorsal mesentery. (It may be mentioned incidentally that in *Polypterus* the hind portion of the large right lung retains throughout life this relatively primitive position in the substance of the mesentery.)

The dorsal mesentery undergoes a remarkable process of thickening from side to side, forming a broad mass of spongy connective-tissue bounded superficially by cœlomic epithelium. It is specially broad dorsally, and it is in this specially broad dorsal region that the lungs are situated, so that as the broadening of the base of attachment of mesentery to dorsal body-wall goes on the originally dorsal part of the mesentery (containing the lungs) becomes gradually completely merged in the roof of the splanchnocœle. The lungs thus come to be situated outside of and completely dorsal to the splanchnocœle.

General Discussion of the Morphology of the Lungs in *Lepidosiren* and *Protopterus*.—I feel compelled to accept the general homology of the organs known in various subdivisions of the Vertebrata under the name of lung and swim-bladder or air-bladder. The early stages of

obviously suggested that the spiral coiling may once have extended forwards into the region of the œsophagus, and that the primary torsion has persisted on the straightening out of the spiral coils.

development of this organ in the ordinary lung-breathing animals, in Lung fishes, and in the most archaic existing Teleostome—*Polypterus*—are identical in their main features, the differences which are so conspicuous in the fully-developed organ of the adult being of a purely secondary character. For convenience I will use the term lung to express the organ alluded to in whatever form it occurs.

It must further, I think, be conceded that the original position of the lung was ventral. This can hardly be denied in view of the fact that in both Crossopterygians and Lung fishes—both of them archaic groups in which the lung plays an important hydrostatic function and has, in correlation with this, assumed in the adult, partially or completely, a position dorsal to the other viscera of the splanchnocœle—the whole lung rudiment is in early stages of development, as is the glottis throughout life, ventral in position.

But, admitting these two points, there at once arises the further question as to the exact method by which the dorsal position of the lung, e. g. of a Teleostean fish, has come about in phylogeny, and this carries with it other questions as to the precise homology of the parts of the dorsal lung of such forms with those of the ventral lung.

It was Sagemehl¹ who first propounded the view which, in its main features, finds a striking corroboration in the facts of ontogeny and adult anatomy of the Dipnoi. Taking the bilobed mid-ventral condition of the lung as a relatively primitive one, Sagemehl points to the condition in *Polypterus* in which the left lobe or left lung has become greatly reduced, as is so frequently the case in lung-breathing Vertebrates with elongated bodies. Were this lopsided condition of the lung carried further, and the left lobe reduced to relatively insignificant dimensions, it is obvious that there would no longer be any insuperable difficulty in imagining a dorsalward shifting of the remaining right lung round the right side of the œsophagus, so that eventually a dorsal position might be attained, as in e. g. *Ceratodus*. Sagemehl

¹ 'Morphol. Jahrb.', x. 1885, p. 108.

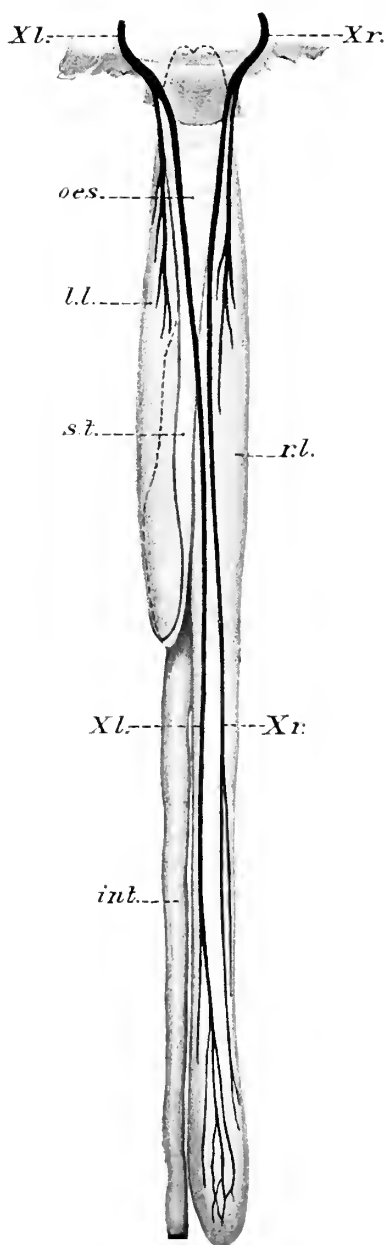
believed that the arrangement in Lung-fishes had actually come about in this way, and that in Actinopterygians matters had gone a step farther in that the glottis had become dorsal as well as the lung itself. Sagemehl, it is true, greatly impressed by the discovery that in *Erythrinus*, *Macrodon*, and *Lebiasina* the glottis was situated on the left side of the pharynx, took the view that in the Actinopterygians the migration of lung apparatus had been up the left side of the pharynx instead of up the right side, as in Lung-fishes. No one, however, who is familiar with the wide range of variation in the position of the glottis, now to the right, now to the left side of the median plane in e. g. Characinids and Siluroids¹ will probably see any reason to suppose that the lung migration has not taken place in exactly the same fashion in the Actinopterygians and in the Lung-fishes.

The Lung of *Polypterus*.—The condition of the lung in the adult *Polypterus* (text-fig. 11, A) must be noticed, especially in regard to three points. Firstly, the lung is here an important hydrostatic organ, and correlated with this we find that the lung apparatus as a whole shows a symmetrical arrangement. The hinder half of the large right lung being without any fellow on the left side to balance it, has assumed a median position lying in the dorsal mesentery, and is practically symmetrical about the mesial plane;² it is only in its anterior half, where it is still balanced by the remains of the left lung, that it bends away from the mesial plane towards the right. It is obvious then that, were the left lung to undergo still further reduction, we should expect more and more of the right lung to assume the mesial position, until at last, when the left lobe had approached vanishing point, the right lobe would tend to be symmetrical about the mesial plane right to its front end. The whole lung apparatus

¹ More especially if we bear in mind the fact, chronicled above, of the definitely dorsal side of the lung in the young lung-fish being carried temporarily to the left side by the primary torsion.

² In the dissection shown in text-fig. 11 A the hinder part of the right lung has been displaced towards the right side and the symmetry is consequently destroyed.

TEXT-FIG. 11 A.



TEXT-FIG. 11 B.



TEXT-FIGS. 11 A, B.—View from the dorsal side of the lung of *Polypterus* (A) and *Lepidosiren* (B) to show nerve supply. *int.* Intestine. *l.l.* Left lung. *l.p.a.* Left pulmonary artery. *oes.* Oesophagus. *ph.* Pharynx. *r.l.* Right lung. *st.* Stomach. *r.p.a.* Right pulmonary artery. *Xl.* Pulmonary branch of left vagus. *Xr.* Pulmonary branch of right vagus.

would thus become medio-dorsal except the pneumatic duct or trachea, which would lead round the right side of the œsophagus or pharynx to the still ventral glottis.

(2) Correlated with the increased size of the right lung in *Polypterus* we find that the left vagus takes a share in its innervation, a stout, in fact the main, branch of this nerve passing across to the right lung dorsal to the œsophagus.

Bearing in mind the mode of development of the lung from a ventrally placed rudiment it need hardly be pointed out that this dorsally placed connection between left vagus and right lung must be a secondary development. How it has come about does not concern the argument, but it may well have been owing to the short circuiting of nerve impulses through the nerve plexus of the pharyngeal wall having caused that part of the plexus which formed the path of the impulses to become enlarged so as to form a distinct nerve trunk.

(3) The glottis or opening from pharynx is no longer symmetrical in the adult *Polypterus* in relation to the two lungs. It has undergone a shifting towards the right side, and is in line with the larger right lung.

The two first of the three points just established have an important bearing upon the comprehension of the conditions seen in the Lung-fishes. (1) shows us how the condition of the Lung-fishes with their dorsal lung communicating with a ventral glottis round the right side of the alimentary canal is just a natural step beyond the condition actually existing in *Polypterus*. (2) does away entirely with the at first sight apparently insuperable objection to adopting this as a phylogenetic hypothesis involved in the peculiar nerve supply of the lung in the Lung-fishes—where (text-fig. 11, B) the pulmonary branch of the left vagus extends on to the actually right lung by a path which is dorsal to the œsophagus—for we see that in *Polypterus* (in which the lung retains the assumed ancestral condition) there already exists a similar prolongation of the left vagus dorsal to the œsophagus to the

lung on the right side of the body, an arrangement that must necessarily have been developed secondarily.

It will be seen that neglecting the discordant evidence of the left vagus as we are now justified in doing, the course of the right vagns, right pulmonary artery, and left pulmonary artery agree in testifying that the lung has undergone a twisting on its long axis in a counter clockwise direction (seen from behind) during its movement to the dorsal position. The X-like crossing of the two pulmonary nerves would of course indicate that this twisting of the lung apparatus had taken place previous to the establishment of the nervous "short circuit" dorsal to the œsophagus.

Having now shown (1) the clear probability of an ancestral arrangement like that of *Polypterus* becoming evolved into an arrangement like that of existing Lung-fishes, and (2) that the obstacle formed by the course of the left vagus is of no importance, it remains now to pass back to the evidence afforded by the ontogeny of *Lepidosiren* and *Protopterns*.

These embryological phenomena show clearly (1) that the lung rudiment is originally ventral in position; (2) that during its development distinct twisting of the lung in a counter-clockwise direction takes place; and (3) that during early stages of development the actual right lung, i.e. the lung which on Sagemehl's hypothesis is homologous with the small left lung of *Polypterus* is actually much smaller than its fellow.

Taking into account these various considerations we are, I think, irresistibly driven to the conclusion that, so far as regards the Dipnoi, Sagemehl's hypothesis must be accorded a very high degree of probability.¹

Actinopterygii.—The support to Sagemehl's hypothesis which has been adduced in the foregoing paragraphs lends increased probability to a similar view being applicable to the *Actinopterygians*. It has been indicated how in *Poly-*

¹ In this I agree with NEUMAYR, Semon's 'Zoolog. Forschungsreisen,' I, p. 407.

pterus the lung apparatus is in process of attaining to a mediodorsal position, the hinder half of the large right lung having already done so. In *Ceratodus* the whole of the lung except glottis and air duct has attained to the mid-dorsal position, and the original left lobe has apparently been completely withdrawn into the lung, so that the latter is a single structure without any paired appearance. It is clearly but a step from the *Ceratodus* condition for the air duct to become shortened and the glottis to reach the neighbourhood of the mesial plane dorsally, such dorsalward migration of the glottis being aided by the tendency of the gut to become rotated in a counter-clockwise direction, as seen from the tailward end.¹

V. PANCREAS.

There are in the *Dipneumona*, as in *Ceratodus* and the majority of Vertebrates, three pancreatic rudiments, one dorsal and two ventral.

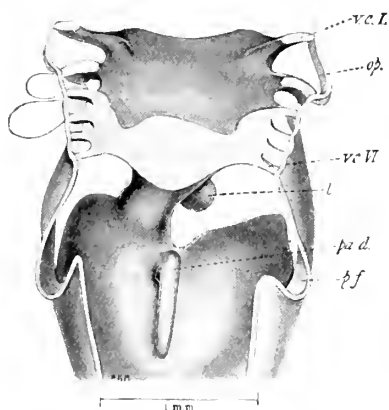
Protopterus.—The dorsal rudiment is the first to make its appearance (about stage XXXII) in the form of a solid projection from the dorsal surface of the yolk practically in the mesial plane. In some embryos of this stage the dorsal pancreatic rudiment is greatly elongated in an antero-posterior direction—possibly a reminiscence of some unknown earlier phylogenetic condition (text-fig. 12, A). In most embryos, however, the dorsal pancreas is of compact and rounded form (cf. text-figs. 8, B, and 12, B), and is situated in about the same transverse plane as the hinder nephrostome of the pronephros. The attachment of the dorsal pancreas becomes rapidly constricted to form a narrow stalk, and a small, irregular cavity appears in the interior of the organ.

By stage XXXIII the ventral rudiments have made their appearance in the form of a yolky projection from the gut on

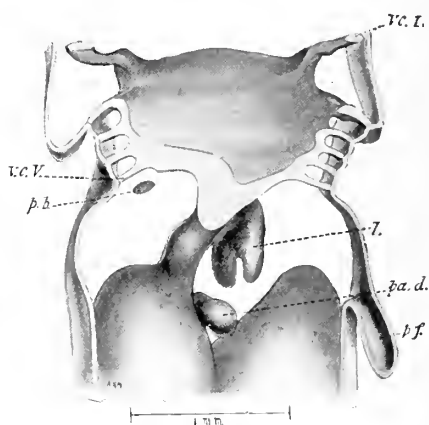
¹ Moser ('Arch. mikr. Anat.,' lxiii, 1904, p. 562) has demonstrated the existence of this in ontogeny. See also the interesting paper by H. Marcus ('Arch. mikr. Anat.,' lxxi, 1908), who is led by his studies on the lung of *Gymnophiona* to a similar conclusion regarding the lung of *Ceratodus*.

either side of the attachment of the bile-duct rudiment. The right is more bulky than the left. Both right and left rudiment grow rapidly in a dorsal direction, one on either side of the bile-duct, and finally the left arches towards the right side, dorsal to the bile duct, comes in contact with the tip of the right rudiment, and undergoes fusion with it (between stages XXXIII and XXXIV most usually). The

TEXT-FIG. 12 A.



TEXT-FIG. 12 B.



TEXT-FIG. 12 A, B.—Reconstruction of thick horizontal slice through *Protopterus*, showing rudiments of lung and (dorsal) pancreas. A. Stage XXXII. B. Stage XXXIV. *l.* Lung. *op.* Operculum. *pa. d.* Dorsal pancreas. *p. b.* Post-branchial body. *p. f.* Pectoral limb. *v. c.* Visceral cleft rudiment.

dorsal pancreatic rudiment has meanwhile been growing rapidly. Its wall is still thick and yolk-laden, but its cavity has increased in size, and has become more regular in shape. With the gradual modelling of the surface of the gut a change has been brought about in the attachment of the dorsal pancreas; it now springs from the right side of the gut just where the mid-gut is continued forwards into the fore-gut. It projects towards the right side of the body, and at the same time slightly forwards.

Up till now the dorsal pancreas has been separate from the

fused ventral rudiments, but between stages XXXIV and XXXV the dorsal surface of the right ventral pancreas comes in contact with the ventral surface of the dorsal pancreas, and fusion promptly takes place, so that from now onwards there is a single pancreatic complex.

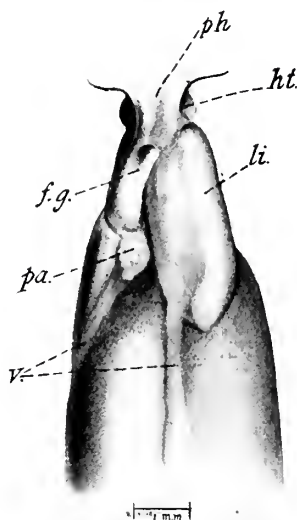
By stage XXXV the pancreatic complex forms a voluminous organ, the dorsal end of which is visible in a dissection from the dorsal side (cf. text-fig. 13 of *Lepidosiren* at this stage), lying immediately to the left of the dorsal lobe of the liver—between it and the junction of fore- and mid-gut, and extending back in the spiral groove. This dorsal part of the complex extends ventrally downwards into the posterior limb of a saddle-shaped mass, which bestrides the bile-duct, and which represents the fused right and left pancreatic rudiments, the original right rudiment having now become posterior in position. The threefold origin of the pancreas is still betrayed by its three attachments to the gut, the original right and left ventral being now immediately posterior and anterior to the point of junction of bile duct and gut, while the long fine duct, which represents the long drawn-out stalk of the dorsal pancreas, opens in the posterior angle between fore-gut and mid-gut. The right and left ventral attachments are still solid. The substance of the gland itself is seen in sections to be undergoing obvious histological differentiation, and to be penetrated by a rich network of blood-vessels.

By stage XXXVI the pancreas has become actively functional, and its cells have attained their definitive character with nucleus at outer end, and the main mass of the cell protoplasm being packed with zymogen granules. With the tucking in of the gut wall to form the spout-like pyloric valve, the opening of the dorsal pancreatic duct is carried inwards, and is now found to open into the cavity of the spout-like structure, the actual opening being on the dorsal wall of the spout.

Lepidosiren.—In *Lepidosiren* the general features of pancreas development are as in *Protopterus*, with minor

differences in topographical relations in early stages. In a larva of stage 32 (text-fig. 4, A) the dorsal pancreas forms a rounded projection from the gut, as in *Protopterus*, but situated farther back, well behind the level of the hinder pronephritic nephrostome. The rudiment in *Lepidosiren* has already a wide cavity opening into the cavity of the gut. By stage XXXIV the yolk in the rudiment is practically used up, and by stage XXXV the gland cells and duct lumina are distinguishable; the whole organ is penetrated by a rich network of blood-vessels, and the outer surface of the organ presents a distinctly lobed appearance (see text-fig. 13).

TEXT-FIG. 13.



Part of the dissection shown in text-fig. 4, B (p. 490), with the lungs removed so as to show the pancreas. *f, g.* Fore-gut. *ht.* Heart. *li.* Liver. *pa.* Pancreas. *ph.* Pharynx. *v.* Vein.

As regards the later development of the pancreas in *Lepidosiren* and *Protopterus*, the only point requiring special notice is that in the adult (cf. text-fig. 4, D), as was

shown by W. N. PARKER, it remains enclosed within the splanchnic mesoderm ensheathing the gut, neither bulging into coelom nor spreading in the substance of the mesentery. As a consequence the pancreas remained undetected by the earlier investigators.

VI. SUMMARY.

1. The fore-gut first becomes folded off from the main mass of yolk-cells.

2. The pyloric valve arises by the hind end of the fore-gut being pushed back into the cavity of the mid-gut.

3. The main mass of yolk-cells becomes gradually "modelled" into a spirally-coiled intestinal rudiment.

4. The main part of the buccal lining is developed in situ from large yolk-cells.

5. The part of the ventral side of the head, on which are the olfactory rudiments, becomes enclosed in the buccal cavity by the development of the upper lips and by the forward growth of the lower jaw.

6. The olfactory opening becomes divided into anterior and posterior nares by the apposition and fusion of the intermediate portion of its lips.

7. The thyroid arises as a solid downgrowth from the buccopharyngeal floor, which gradually becomes cut off from behind forwards.

8. The tongue is a primary tongue like that of Urodeles, but without gland-field.

9. The lung arises from a solid mid-ventral rudiment.

10. When the lung becomes bilobed, the (actual) right lobe is for a time small in size as compared with its fellow.

11. Complicated torsional processes take place during the development of the lung.

12. Through the dorsal mesentery becoming partially merged in the splanchnocœle roof, the lungs come to lie outside the splanchnocœle.

13. The general facts of lung development go to support the view that the lung of *Polypterus* shows a persistence of the condition ancestral to that of *Dipnoi* and *Actinopterygii*.

14. The pancreas arises from a dorsal and two ventral rudiments.

The Phylogeny of the Tracheæ in Araneæ.

By

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With Plate 28, and 21 Text-figures.

INTRODUCTION.

IN an excellent paper on the tracheæ of spiders E. Lamy (:02) has given an account of the tracheæ of thirty families of Araneæ, so that only four small and comparatively rare families, comprising 1—3 genera each, remain, of which the tracheæ are still unknown. It is now possible, therefore, to consider the tracheal systems of the Araneæ as a whole from a phylogenetic point of view, and as I barely touched upon this point in my paper (:09) on the development and origin of the respiratory organs in spiders, I propose to make it the subject of the present paper.

Lamy has made it perfectly clear that the degree of complication of a tracheal system, as regards the manner and extent of the branching and the structure of the internal armature (spines, spiral thread, etc.), cannot be used as a family character, since we may find the most varied degrees of complication amongst the different genera of one and the same family (e.g. in the Uloboridæ, Thomisidæ, Agelenidæ, Clubionidæ, Attidæ, etc.). Lamy concludes from this that the tracheal apparatus is evolved separately in each family and not in the Araneæ as a whole (p. 265) and this statement may, I think, be accepted as in general correct,

provided that it be not interpreted to mean the development of the tracheæ out of lung-books separately in each family but merely the development of more complicated tracheal systems from simpler ones or *vice versâ*.

Another important point which Lamy has emphasised (p. 264) is that the number of the lung-leaves is in inverse ratio to the size of the tracheal apparatus. Thus, forms with highly-developed tracheæ have a small number of lung-leaves, as *Dictyna*, with 4—5 leaves (Bertkau), *Segestria*, with 10—12 (Bertkau), etc., while forms with a feebly-developed tracheal system have a comparatively large number of lung-leaves, as *Epeira* and *Agelena*, with 60—70 leaves (Bertkau).

Since the relative size of the tracheal apparatus in general increases proportionately with its degree of complication it further follows from the above paragraphs that the number of the lung-leaves in forms having both tracheæ and lung-books can have no greater phylogenetic value than that possessed by the degree of complication (in respect to branching and internal armature) of the tracheal apparatus and this Lamy has shown to be of subordinate phylogenetic value, without even the importance of a family character. We cannot, in fact, use either of these characters in comparing widely remote families phylogenetically, as, for instance, the *Dysderidæ* and *Argiopidæ* (*Epeiridæ*). Thus, assuming that the tracheæ were derived from lung-books, it would be incorrect to argue that an *Argiopid*, with its numerous lung-leaves and simple tracheæ is, on that account, a more primitive form than a *Dysderid*, with its few lung-leaves and large complicated tracheæ; or, conversely, if we admit that the *Dysderidæ* are more primitive than the *Argiopidæ*, we could not argue that because of the different relative development of these two organs in the two families, the lung-books must have been derived from tracheæ.

It does not follow, however, from any of Lamy's arguments, that the two characters just discussed are of no phylogenetic importance at all, e. g. amongst allied genera in one and the

same family, as in the Agelenidæ, or between allied families, such as the Dysderidæ and Oonopidæ—nor that other tracheal characters, such as the acquisition of the respiratory function by the ectodermal tendons of the tracheal segment, which I have shown to have taken place in most spiders, may not have a much higher phylogenetic value.

Before entering upon this subject I wish to consider a certain remarkable conclusion drawn by Lamy, viz. that in spiders neither the lung-books nor the tracheæ are the more primitive organs (p. 264), both having been produced simultaneously and replacing one another (p. 265). The sole difference Lamy sees between these two organs lies in their special mode of branching, lamellate branches producing lung-books and tubular ones tracheæ (p. 267). The branchial origin of the lung-books is discarded by him as unnecessary and the formation of the tracheal organs is considered to be the consequence of the respiratory function taking place in the same conditions in all air-breathing Arthropods (pp. 266 and 267).

Lamy arrives at the above conclusion by the following arguments (p. 264):—(1) The Dysderidæ and the Caponiidæ come very near together, approaching one another in several characters, and ought, therefore, to be regarded as equally primitive. Nevertheless, in the latter, the first pair of lung-books is replaced by a pair of tracheæ, which strongly resemble those which replace the second pair of lung-books in the Dysderidæ. The fact that one sees the tracheæ indifferently replacing the lung-books in these two somewhat primitive families indicates that neither organ is to be regarded as more primitive than the other. (2) The same conclusion results from the fact that we find amongst the *Aranææ veræ*¹ another family, the Hypochilidæ, which,

¹ Simon divides the spiders as follows: *Aranææ theraphosæ* (= *Mygalomorphæ*, Pocock + *Liphistius*), including all 4-lunged forms except the Hypochilidæ. *Aranææ veræ* (*Arachnomorphæ*, Pocock), including the Hypochilidæ and all dipneumonous and apneumonous spiders.

although it resembles the *Araneæ veræ* and not the *Theraphosæ* in all other respects, nevertheless, has the tracheæ replaced by a second pair of lung-books.

Neither of these arguments, however, warrant the conclusion that Lamy has drawn from them, since both cases may be readily explained, even when we assume that the lung-books were in all cases the primitive organ and that the tracheæ were derived from them. Lamy's assumption that the *Dysderidæ* and the *Caponiidæ* are equally primitive is certainly incorrect, since, as I shall presently show, the *Caponiidæ* differ from the *Dysderidæ*, as well as from all other spiders (so far as I know), in several important anatomical characters. They are in fact, in these respects, a highly-specialised group, compared with which the *Dysderidæ* are much more primitive. But even if these two families were equally primitive they are by no means the most primitive spiders, the vast host of mygalomorphous forms being all more primitive than they and all provided with lung-books only. Moreover, the highly-developed tracheæ of the *Dysderidæ*, which present no obvious resemblance to lung-books, do not so strongly resemble the anterior tracheæ of the *Caponiidæ* as Lamy makes out, since these latter are very similar to the lung-books of a *Dysderid*. In fact, these anterior tracheæ may be most readily explained, as I shall presently show, as lung-books which have been transformed into tracheæ more recently than those of the second pair and which have retained the primitive shape more nearly than has been the case with the tracheæ of any other Arachnid known. They have evidently been evolved out of a few-leaved lung-book like that of a *Dysderid*, and their presence merely proves that tracheæ have been evolved out of lung-books within the *Araneæ* at least on two occasions, but it does not prove that the tracheæ and lung-books are equally primitive.

Similarly, the presence of a second pair of lung-books in the *Hypochilidæ* may be quite readily explained by assuming that this family is an arachnomorphous form in

which the primitive lung-books have been retained, whereas they have been replaced by tracheæ or lost in all other members of the group. It is not at all necessary to assume that the Hypochilidæ once possessed tracheæ, nor that the lung-books and the tracheæ must necessarily be equally primitive organs.

Lamy's conception of a lung-book as developing from an ectodermal invagination with lamellate branches (p. 256) is incorrect, since, as I had already shown some years previously ('95), the two oldest sacculi¹ are formed as independent invaginations on the free posterior side of an embryonic abdominal appendage, quite outside of the basal pulmonary sac (vestibule) in the anterior wall of which the remaining sacculi appear,—the two oldest sacculi being only later on included within the pulmonary sac, when the sinking of the appendage takes place. Lamy puts this observation aside with the remark that I am the only observer who mentions it, but it is none the less a fact.

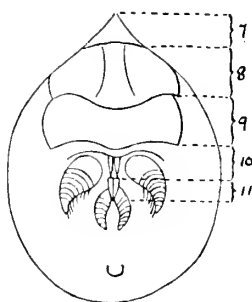
I have already (:09) fully discussed the question of the primitiveness of the lung-books, and have shown on purely embryological grounds that the typical form of tracheæ found in most spiders must have been derived in part from lung-books and in part from ectodermal tendons (entapophyses of Ray Lankester, apodemes), the lateral pair of tracheal trunks being metamorphosed lung-books and the medial pair metamorphosed entapophyses.

Starting from this as a basis, the tetrapneumonous group, Araneæ theraphosæ, appears the most primitive of living Araneæ, a view which has, in fact, long been generally recognised on account of other primitive characters of the group, such as the presence of a free nervous ganglion behind the central nervous mass in the cephalo-thorax, the

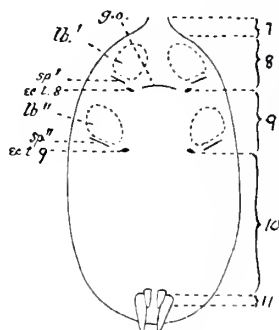
I have given the term "sacculi" to the hollow air-containing leaves of a lung-book and "septa" to the partitions or lamellæ separating the cavities of adjacent air chambers (:09).

simple form of the external sexual organs, the presence of four spiracles, etc.

The remarkable genus *Liphistius*, which I have had no opportunity of carefully examining, appears, as Pocock ('92) has pointed out, to be much more primitive than the rest of the group, at least in some of its characters. One of the most interesting among these is the mesial position of the spinners on the under side of the abdomen, so that in this genus none of the ventral abdominal segments have been excessively elongated (text-fig. 1). Considering the apparently primitive structure of this genus, which has also its abdomen



1.



2.

TEXT-FIG. 1.—Abdomen of *Liphistius* (after Pocock, '92).

TEXT-FIG. 2.—Abdomen of a mygalomorphous spider.

ec. t. 8 and 9. Depressions in the integument to which the ventral longitudinal muscles are attached at the posterior margins of somites 8 and 9. *g. o.* External genital opening. *lb.*, *lb''*. First and second pair of lung-books. *sp'*, *sp''*. Spiracles of the first and second pair of lung-books. 7—11 denote the extent of the seventh to eleventh somites.

segmented dorsally like a Pedipalp, it is somewhat peculiar that both respiratory segments (judging from the figures and descriptions given by Simon and Pocock) evidently possess a deep interpulmonary or epigastric fold, like the Pedipalpi and the *Araneæ veræ*.

In the rest of the group (Pocock's *Mygalomorphæ*) the fourth abdominal (tenth post-oral) segment has greatly

elongated at the expense of the following segments, being, in fact, as long as or longer than the second and third segments taken together, so as to bring the spinners to the hinder end of the abdomen (text-fig. 2). The positions of the spiracles (*sp'*, *sp''*), muscular stigmata (*ec. t.* 8 and 9, representing rudimentary entapophyses to which the longitudinal muscles are attached), and the genital opening (*g. o.*) are very primitive, at least in all the forms which I have been able to examine. All these openings are frequently perfectly exposed and separate from each other, especially in distended abdomens, as in text-fig. 2. There is at most a shallow, open, transverse depression behind the posterior edge of the segments, and the skin in this groove behind the genital segment is frequently soft and flexible, like the soft skin between the hard plates of a segmented body. When the abdomen is distended the spiracles and muscular stigmata in this soft skin are exposed, but in a contracted abdomen (such as that of a female after the deposition of the eggs) these openings may become somewhat hidden from view owing to the infolding of the flexible skin. Such a groove is, however, very different from the typical, deep, and more or less rigid infolding found behind the second and third abdominal segments in the Pedipalpi (see Taruani, '89, p. 377, fig. 1, and Lankester, :04, fig. 5*b*), nearly all arachnomorphous spiders, and in Liphistius. The longitudinal muscles are attached to shallow ectodermal depressions (see my paper, :09, fig. 36), which lie either free or in the larger transverse grooves mentioned above, and there are, so far as I know, no deep invaginations or ectodermal tendons (entapophyses) like those found in Pedipalps and arachnomorphous spiders. Can this and the absence of interpulmonary folds perhaps be a secondary condition in the Mygalomorphæ? Or have these folds been acquired independently in the Pedipalpi, Liphistius, and Arachnomorphæ¹?

¹ Two interesting drawings by R. I. Pocock are given by Ray Lankester (:04, figs. 56 and 64) showing the genital segments of a male *Thelyphonus assamensis* and a female *Liphistius de-*

Turning now to the *Araneæ veræ* or *Arachnomorphæ*, we find that in nearly every case the second pair of respiratory organs (and in one family the first pair as well) have been replaced by tracheæ, the exceptions being the small family *Hypochilidæ* with two pairs of lung-books and the *Pholcidæ*, in which there are no other respiratory organs besides the single pair of lung-books of the genital segment. Moreover, there is a deep infolding along the hind edge of each of the respiratory segments between the spiracles, so as to hide from view the genital opening and the external openings of the well-developed ectodermal tendons or entapophyses of the ventral longitudinal muscles. There are a

sultor, with the epigastric fold drawn apart so as to expose the genital opening and the edges of the septa of the lung-books. These two figures are remarkable for showing that in these two *Arachnids* the pulmonary saccules of the genital segment open directly into the cleft of the epigastric fold, being, in fact, attached to the anterior wall of the fold. I examined a female of *Thelyphonus caudatus*, and found the conditions exactly as depicted by Pocock. The pulmonary ante-chamber opens along its entire medial side into the median part of the epigastric fold, and cannot, therefore, be said to form a separate chamber, except in its dorso-lateral prolongation or portion containing the youngest saccules. In the male (i. e. specimens with a spine on the second abdominal sternite, teste Kraepelin) of this species, however, I found the conditions different. Here there is a longitudinal fold of integument on each side between the deep median part of the epigastric fold and the anterior pulmonary chambers, so that the latter may be said to form separate chambers opening by the ventral slit only into the epigastric fold, as is usual in dipneumonous spiders. The condition depicted by Pocock in *Liphistius* is not known to occur in any dipneumonous spider, and may indicate that the epigastric fold of this form is directly connected with that of *Thelyphonus* and not of independent origin, in which case the absence of the fold in the *Mygalomorphæ* would be a secondary condition. Its presence in the four-lunged arachnomorphous family *Hypochilidæ* is also an interesting circumstance.

These two figures of Pocock's should have been included in the historical list of papers concerning the lung-books of *Arachnids* given at the end of my previous paper (:09). They unfortunately did not come to my notice until after the paper had been sent to the press.

few rare exceptions—thus in the *Dysderidæ* the tracheal segment has no infolding and the genital duct sometimes opens free on the ventral surface of the pulmonary segment (male of *Harpactes*; see my paper :09, fig. 40).

Leaving the four-lunged *Hypochilidæ*, with which I am unacquainted, out of account, there appears in the first place a small group of three families (*Dysderidæ*, *Oonopidæ*, and *Caponiidæ*) which possess some very primitive features in connection with their respiratory segments. As these segments are of peculiar interest in connection with the phylogeny of the tracheæ, I shall give some account of their anatomy before proceeding to more general conclusions.

Material and Treatment.—The material used was the same as that given in my previous paper (:09) with the addition of specimens of a species¹ of *Oonopidæ* from the neighbourhood of Cape Town.

For following the muscles, which are often very slender, suitable differential staining is very necessary, and for this purpose I found very old Delafield's hæmatoxylin (mine was thirteen years old) most excellent, even for old museum specimens. The sections are stained on the slide for four to five hours, placed in acidulated alcohol for three to four

¹ As this species is a new one, I append the following description: *Calculus* n. g. Cephalothorax broadly ovate. Ocular area transverse, the eyes arranged as in *Orchestina*. Labium short and broad, as in *Oonops*. Coxæ of pedipalps parallel, their anterior ends widely separated and not converging. —*C. bicolor* n. sp. Pale yellowish, abdomen with a broad infusate patch behind above, and narrowly blackened on each side of the spinners as well. Clypeus barely as wide as an anterior lateral eye. Anterior row of eyes, seen from above, almost straight, the median eyes large, a trifle longer than their distance from the anterior margin of cephalothorax; anterior lateral eyes the smallest of the six, distant about half their own width from the median eyes; posterior eyes forming a row which is only very slightly wider than the anterior row, their distance from the median eyes greater than their own width. Tibia and metatarsus of first leg with 0-2 spines near the middle below, tibia and especially the metatarsus of fourth leg more numerous spined. Several females from the Cape Flats, near Princess and Zeekoe Vleis. Length 4 mm. Allied to *Telchius*, E. Sim.

minutes, washed with spirits, and then held inverted over a vessel containing a drop of ammonia in some water until the sections change colour. After this they should be mounted in balsam without delay. The nuclei become blue and the muscles reddish and easily distinguishable from other tissues.

In preparing the tracheæ by the caustic potash method I obtained the best results for such highly complicated systems as those of *Caponia* by first allowing the object, after removal of a part of the dorsal integument, to remain in cold concentrated caustic potash for twelve hours or longer. If the solution be then gently heated and some water added the soft parts remaining will rapidly disappear without injury to the delicate tracheæ. These should be examined in water or weak alcohol, and not in glycerine or acetate of potash, since these latter cause the tubes to collapse and become distorted.

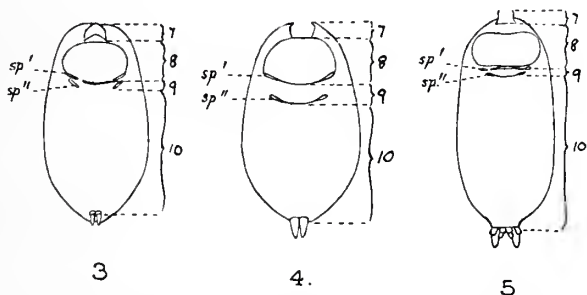
THE RESPIRATORY SEGMENTS OF THE DYSDERIDÆ, OONOPIDÆ, AND CAPONIIDÆ.

These spiders more nearly resemble the *Mygalomorphæ* than they do the rest of the *Arachnomorphæ* in the anterior position and wide separation of the second pair of spiracles (*sp''*., text-figs. 3—5) and the more rudimentary condition of the ectodermal tendons (where present) of the second respiratory segment.

Further, the transverse epigastric fold, lying between the two anterior spiracles, although present, never encloses a spinous canal of communication connecting the lumens of the two anterior respiratory organs, while the *Dysderidæ* are unique amongst arachnomorphous spiders in having no inter-tracheal fold between the two posterior spiracles (text-fig. 3), a primitive character only met with elsewhere in the *Mygalomorphæ* (p. 524, text-fig. 2). Owing to the presence of tracheæ instead of lung-books in the ninth somite this segment is somewhat shortened, but in other respects the

extent of the abdominal segments in the three families much resembles that in the Mygalomorphæ.

Dysderidæ.—The lung-books in the Dysderidæ have few leaves. I counted about thirteen in *Harpactes* (in sections), but there are more in *Dysdera* and *Segestria senoculata* (Bertkau ['72] records only ten to twelve for *Segestria*). The ante-chamber (*pulm. a.*) is strongly inclined forwards from the base at an angle of 40° — 50° , and is evenly curved forwards in *Harpactes* (Pl. 28, fig. 5), but almost straight in *Dysdera* and *Segestria* (p. 530, text-fig. 7). It is spined on its posterior wall except quite inferiorly, where a muscle (text-fig. 7, No. 11) is attached.



TEXT-FIG. 3.—Abdomen of *Dysdera* sp., ad. ♀ (magn. 3).

TEXT-FIG. 4.—Abdomen of *Calculus bicolor*, ad. ♀ (magn. 10).

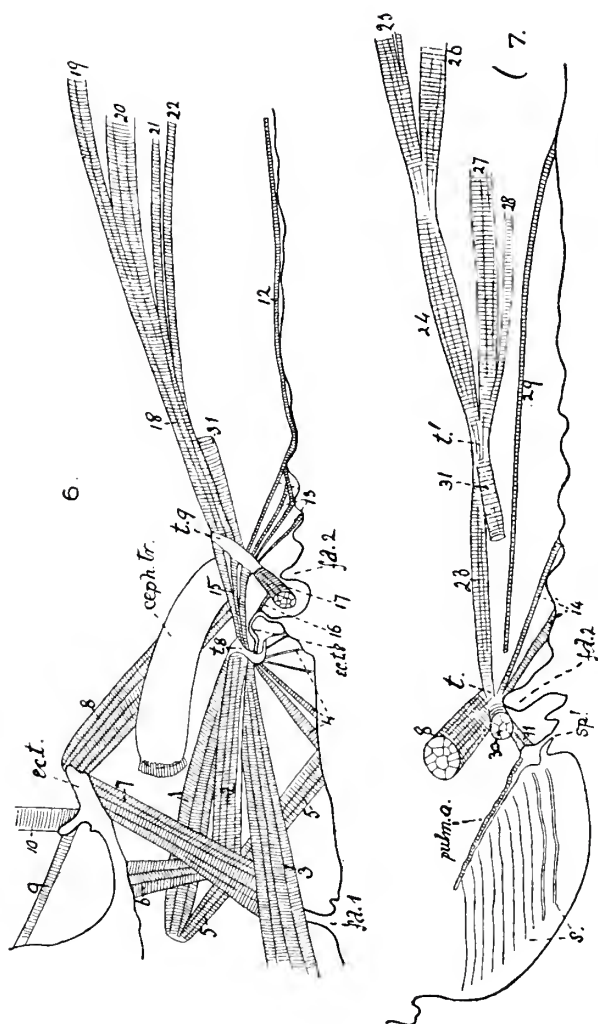
TEXT-FIG. 5.—Abdomen of *Caponia spiralifera*, ad. ♀ (magn. 3).

sp', *sp''*. Spiracles of the first and second respiratory segments.
7—10 denote the extent of the seventh to tenth somites.

The peculiarities of the epigastric fold have already been described (:09).

The well-known tracheæ (p. 551, text-fig. 19) have been described by several authors (see Lamy [:02, pp. 180—183] for some excellent figures of *Dysdera* and *Segestria*), and I have given a summary of their structure with some additional observations on the entapophyses and muscles connected with them (:09).

In order to ascertain anatomically whether a trachea or a



Diagrams of the muscles connected with the respiratory segments of *Segestria senoculata*, ad. ♂ (magn. 40). Text-fig. 7 is more lateral than text-fig. 6, and both are represented as seen from the medial side. *ceph. tr.*, Cephalothoracic trunk of trachea. *ec. t.*, Ectodermal tendon (of the large, anterior, dorsal pair behind the pedicel). *ec. t. 8*, Entapophysis of the pulmonary segment. *fd. 1*, Fold in lower part of pedicel. *fd. 2*, Lateral fold in which the tracheal spiracle lies. *pulm. a.*, Pulmonary ante-chamber. *s.*, Sacculus of lung-book. *sp'*, Spiracle of lung-book. *t.*, *t'*, *t. 8*, *t. 9*, Entochondrites. 1—31, Muscles.

part of one is homologous with a lung-book or with an entapophysis it is necessary first of all to identify the entapophyses of the great longitudinal muscles. These entapophyses, as I have shown for *Attus* and *Agelena* ('95, :09), arise in various abdominal segments as invaginations on the posterior side of the provisional appendages, while the invaginations which form, or correspond to, a pulmonary sac or ante-chamber always lie to the lateral side of the entapophyses. In the two segments bearing the spinners in *Attus* and *Agelena* the entapophyses are attached at the posterior, medial, basal corners of the anterior and posterior spinners. For the identification of the entapophyses anatomically a knowledge of the abdominal muscles connected with the respiratory segments is necessary, and I have given the two accompanying diagrams (text-figs. 6 and 7) to illustrate these muscles and their entochondrites in a typical *Dysderid*.

List of the Entochondrites and Muscles in Text-figs. 6 and 7.

t. Small entochondrite on the lateral side of the trachea and attached to the fold of the integument, *fig.* 2.

t. Entochondrite between the muscles 23 and 24, etc., but not attached to the integument.

t. 8. Large entochondrite situated on the medial side of the pulmonary ante-chamber and attached to the epigastric fold.

t. 9. Corresponding entochondrite of the tracheal segment, situated on the medial side of the trachea.

Muscles.

1 and 2. From the entochondrite *t.* 8 to upper and middle part of side of abdominal pedicel.

3. Longitudinal from the entochondrite *t.* 8 to the cephalothorax.

4. From the entochondrite *t.* 8 to ventral integument of pulmonary segment.

5. From upper part of side of abdominal pedicel to ventral integument of pulmonary segment. (1 and 5 are inserted together anteriorly.)

6. Dorso-ventral on side of abdominal pedicel. (2 and 6 are attached to the same ectodermal infolding on the medial side of 5.)

7. Oblique dorso-ventral from hinder end of the ectodermal tendon *ec.t.* to the ventral fold *fil.1* of abdominal pedicel.

8. Oblique dorso-ventral from hinder end of the ectodermal tendon *ec.t.* to the entochondrite *t.*

9. From the ectodermal tendon *ec.t.* to anterior integument of abdomen.

10. From the ectodermal tendon *ec.t.* to dorsal integument of abdomen.

11. From lateral part of posterior side of pulmonary ante-chamber in a postero-dorsal direction to the entochondrite *t.* (This muscle can widen the ante-chamber in *Segestria*.)

12. Longitudinal parietal along ventral integument of abdomen.

13. From the entochondrite *t.9* to ventral integument of abdomen (some strands apparently continuous with 12.)

14. From the entochondrite *t.* to ventral integument of abdomen.

15. Longitudinal connecting the entochondrites *t.8* and *t.9*.

16. From posterior side of entapophysis (*ec.t.8*) of pulmonary segment to anterior side of the integumental fold *fil.2*.

17. Subtransverse from the entochondrite *t.9* in a medial direction to posterior side of base of epigastric fold.

18. Longitudinal from the entochondrite *t.9* to the spinners, breaking up posteriorly into 19, 20, 21 and 22.

19. Three muscles from 18 (one large one to medial side of large posterior spinner and two smaller ones to small mesial spinner).

20. From 18 to posterior medial side of anterior spinner.

21. From 18 to medial side of same spinner.

22. From 18 to anterior side of same spinner.

23. Longitudinal connecting the entochondrites *t.* and *t'.*
 24. Longitudinal from the entochondrite *t'.* to the spinners, dividing posteriorly into 25 and 26.
 25. Two muscles from 24 to anterior lateral angle of large posterior spinner.
 26. From 24 to posterior lateral angle of anterior spinner.
 27. From the entochondrite *t'.* to anterior lateral side of anterior spinner.
 28. From the entochondrite *t'.* to anterior side of anterior spinner (inserted behind 22).
 29. From posterior end of the cephalothoracic tracheal trunk *ceph. tr.* (inserted just below the origin of the short abdominal branch) to ventral integument of abdomen.
 30. Subtransverse connecting the entochondrites *t.8* and *t.*
 31. Connecting the entochondrites *t.9* and *t'.*
- (Two other muscles of the female of *Harpactes* are given in Pl. 28, fig. 3).

The part of the epigastric fold (text-fig. 6, *ec.t.8*) to which the entochondrite *t.8* is attached, plainly corresponds to the entapophysis of the pulmonary segment in other dipneumonous spiders (see my paper :09, fig. 41, *ec.t.8*), but the identity of the corresponding entapophysis of the tracheal segment is not at first sight so evident, since there are two entochondrites, one on each side of the trachea, and two sets of longitudinal muscles, both connected with the entochondrite *t.8*. In fact, the whole muscular system of the ninth and tenth somites is very different to that of *Attus*, *Agelena*, *Epeira*, etc., which is, of course, due to the circumstance that in the latter the relative lengths of the two somites are exactly reversed. From the fact that the muscles 19—21 of the medial set in *Segestria* (text-fig. 6) are connected with the medial and postero-medial sides of the anterior and posterior spinners, while the muscles 25—27 of the lateral set (text-fig. 7) are connected with the lateral side of the same spinners, it is evident that the entochondrite *t.9* is the one which in the tracheal segment corresponds to the entochondrite *t.8* of the pulmonary segment. The

entochondrite *t.9* is attached (in *Segestria* at least) to a small lobe of the medial side of the tracheal pedicel. This lobe (the entapophysis of the tracheal segment), which I have already described and figured (:09, figs. 32 and 33, *ec.t.9*), is not really a part of the trachea proper, being merely connected with the base of the pedicel and unspined internally, and I am not even sure that it is present in *Dysdera* or *Harpactes*, in which genera the entochondrite may possibly be attached directly to the integument at the medial basal angle of the tracheal pedicel. From the position of the trachea on the lateral side of the entochondrite *t.9*, it is evident that the whole of the trachea must be considered as homologous with a pulmonary sac or lung-book, as I have already pointed out ('95) and Lamy also agrees with this view by reason of the position and appearance of the tracheæ and the complete separation of the spiracles (:02, p. 259).

Oonopidæ.—This family is very closely allied to the *Dysderidæ* as was pointed out by Bertkau ('78), who included *Oonops* in the latter. The only anatomical difference of any importance connected with the respiratory segments appears to be the presence of an intertracheal fold observed by Lamy (:02), joining the two tracheæ in the *Oonopidæ*.

I found the muscular system connected with these segments to be practically identical in the two families, all the characteristic muscles of the *Dysderidæ* being present in the *Oonopid*, *Calculus bicolor*, examined by me, although sometimes in a somewhat modified form.¹ Thus the muscle 29 (text-fig. 7) is much shorter and 15 (text-fig. 6) somewhat longer in the *Oonopid*, as the posterior tracheæ are here placed further back. The lateral entochondrite *t.* is particularly conspicuous, much more so than in *Segestria*.

The tracheal trunks are quite similar in both families. Those of *Oonops* were first described by Bertkau ('78) and later in greater detail by Lamy (:02), who also examined a

¹ Three additional muscles not noticed in *Segestria* are given in figs. 1 and 2, but two of these (*m.* 38 and 40) are also found in the female of *Harpactes* (fig. 3).

Dysderina. Neither of these authors, however, observed the anterior ending of the cephalothoracic trunks, but quite correctly supposed them to end, as in the *Dysderidæ*, in a bundle of tubules. In *Calculus bicolor* these trunks are short and very much as in text-fig. 6. They do not enter the abdominal pedicel, but break up at the anterior end into a dense cluster of fine tubules, which then pass through the pedicel into the cephalothorax. The short posterior branch, first found in this family by Lamy, is also present, and corresponds exactly to the similar branch in the *Dysderidæ*. The anastomosing ends of the internal spines form a simple network, like that in *Harpactes*, but in the forms examined by Bertkau and Lamy they are said to form a spiral thread. The cavities of the two tracheal trunks are directly connected by a spinous canal of communication (fig. 1, *can.*), enclosed within the intertracheal fold (*tr. fl.*). As in the *Dysderidæ*, the tracheal trunks and their branches are to be considered as entirely homologous with lung-books.

Another important point of resemblance to the *Dysderidæ* is the presence in the female of a single median receptaculum seminis, pointed out by Bertkau ('78), who observed that such a receptaculum is not found in any other family of spiders besides these two (p. 374). In *Calculus bicolor* the receptaculum forms an elongate, narrow, curved, median pouch (fig. 2, *r. s.*), placed horizontally with the concavity of the curvature upwards, and opening into the anterior wall of the epigastric fold (*ep. fl.*). From the under side of the pouch a large vertical keel (*k.*) hangs downwards, reaching to the body hypodermis. Each side of this keel serves for the attachment of a powerful muscle (*m.* 38), which runs obliquely backwards and outwards to the anterior surface of the entapophysis of the epigastric fold (fig. 1). There is also a median muscle (*m.* 40) running from the under side of the pouch along the posterior edge of the keel to the ventral body integument. In *Harpactes Hombergi* I found a very similar receptaculum, provided with a similar remark-

able keel and pair of muscles (fig. 3). Bertkau ('78) pointed out the similarity between *Oonops* and *Harpactes* as regards their female sexual organs (p. 371), but he does not describe or figure the keel and muscles ('78, pl. xii, fig. 8). In *Segestria* and *Dysdera* the receptaculum seems to be differently formed. (See Bertkau, '75, pl. vii, fig. 12, and '78, pl. xii, fig. 6.)

The lung-book in the *Oonopid* which I examined has nearly twenty leaves. Its ante-chamber differs from that of the *Dysderidæ* and has the normal shape found in many other dipneumonous spiders, that is to say, it rises vertically from its pedicel but soon curves gradually forwards to form a long "horn," which is nearly horizontal in its anterior part. The ante-chamber is densely spined on its posterior wall, except along its lateral edge inferiorly, where a small muscle (corresponding to No. 11 in text-fig. 7, p. 530) is attached.

There is a distinct and deep epigastric (interpulmonary) fold, which ends laterally just behind the medial ends of the pulmonary spiracles, but is not continuous with them. There is, therefore, no canal of communication between the lung-books and no part of the fold is lined with spines (see fig. 2). As in *Dysdera* and *Segestria*, the portion of the fold between the entochondrites is much deeper than the portions which lie laterally to these. The lateral corners of this deepened part of the fold form the entapophyses to which the entochondrites (fig. 1, *t. 8*) of the ventral longitudinal muscles are attached. These entapophyses are somewhat unusual in form, their deeper part forming a solid, darkly staining plate (fig. 1, *ec. t. 8*), the anterior face of which serves for the attachment of the obliquely transverse muscle (*m. 38*) connected with the keel of the receptaculum seminis, while the entochondrite (*t. 8*) is attached to the upper lateral edge of the plate.

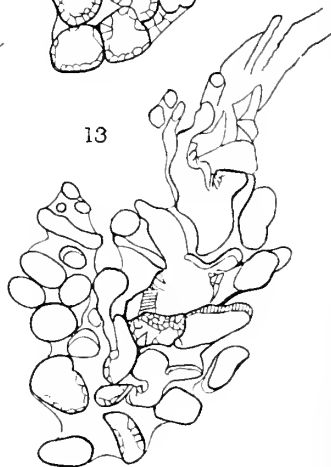
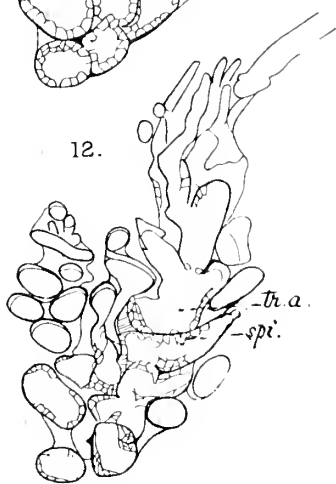
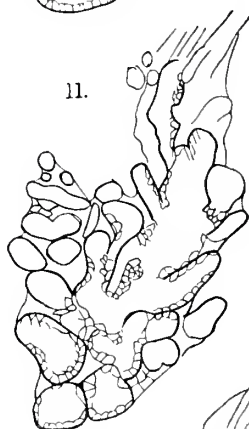
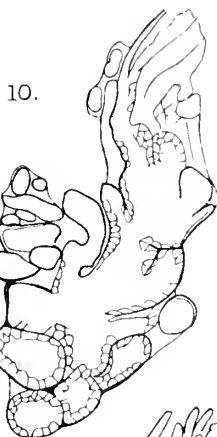
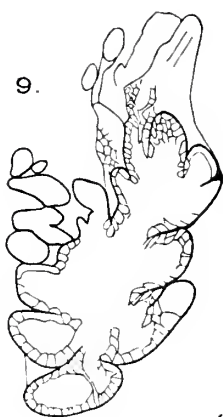
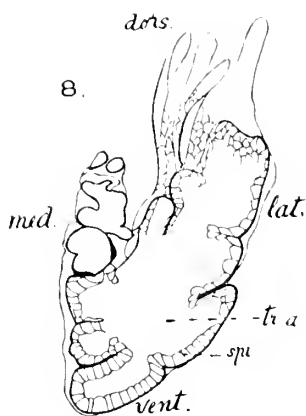
Caponiidæ.—This small but very interesting family bears some external resemblance to the *Dysderidæ* and *Oonopidæ* but it differs from these and, so far as I know, from all other spiders as well, in four unique and remarkable anato-

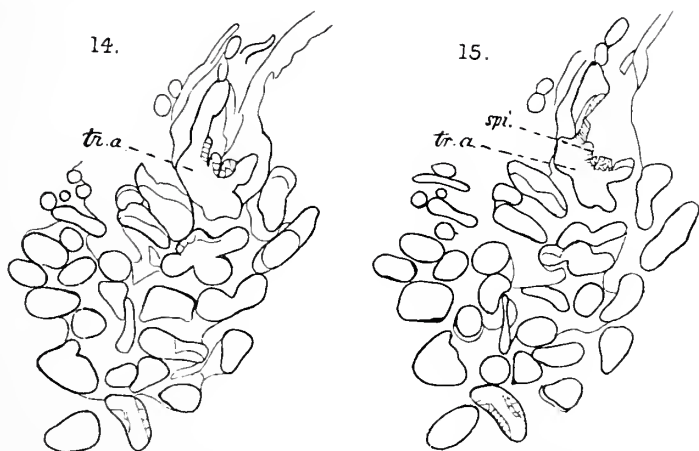
mical characters connected with the respiratory segments. These are (1) the presence of an anterior pair of tracheæ in place of the lung-books (apneumonous spiders), (2) the presence of a peculiar sense-organ within the second pair of tracheæ, (3) the absence of the segments (corresponding to 15 and 18, text-fig. 6) of the great, ventral, longitudinal muscles, so conspicuous in other spiders, belonging to somites 9 and 10 and of the entochondrites connected with them, and (4) the replacement in the female of the usual receptacula seminis of the epigastric fold by a pair of great chamber-like dilations of the oviducts in the upper anterior part of the abdomen.

The tracheæ of *Caponia* and *Nops* have been very well described and figured in Simon ('93, pp. 326 and 327, figs. 294 and 295) from drawings made by Bertkau (also reproduced by Lamy [02, p. 184, figs. 24 and 25]). The following description was made from a number of sections and other preparations of *Caponia spiralifera*, Purc., specimens of which were collected for me at Hanover, Cape Colony, and well preserved in spirits by my friend, Mr. S. C. Cronwright Schreiner.

The anterior pair of tracheæ (p. 545, text-fig. 17, and fig. 9, *a.tr.*) are situated in precisely the same place which is occupied by the lung-books in dipneumonous spiders, and they are evidently merely a pair of lung-books of which the sacculæ have been metamorphosed into branched tracheal tubules. Fig. 10 shows one of these tracheæ, prepared in caustic potash and seen from the medial side. Figs. 11 and 12 are from sagittal sections.

The spineless, thick-walled pedicel (fig. 11, *ped.*), which is continuous with the adjacent epigastric fold and much resembles it in sagittal sections, leads from the spiracle (*sp'*) into an ante-chamber (*tr.a.*). The latter is shaped much like that of the lung-book of a *Dysderid*, being strongly inclined forwards and slightly outwards, broadest near the base and tapering towards the higher anterior end or apex (figs. 10 and 12). It is, however, somewhat more capacious, owing to the





TEXT-FIGS. 8—15.—Eight consecutive transverse sections through an anterior trachea of *Caponia spiralifera*, commencing with the most posterior one of the eight (see Pl. 28, fig. 11). *spi.* Anastomosing spines of ante-chamber. *tr. a.* Ante-chamber. (The lateral side of the trachea is on the right of each figure.) Magn. 192.

greater elongation of its ventral side. Internally the ante-chamber is lined with anastomosing spines (*spi.*), except laterally near the base of the upper (posterior) wall, where there is a fold (*fil.*) to which a short muscle (No. 11 in text-fig. 17, p. 545) is attached, exactly as in the *Dysderidæ*. Nearly all the tracheal tubules, except a medial group of four, spring from the ascending anterior side of the ante-chamber and run forwards. They occupy exactly the position of the saccules of a lung-book (c.f. text-figs. 7 and 17) from which they are plainly derived. In fact, if the 13 or 14 saccules of the lung-book of a *Harpactes* (fig. 5) were each divided longitudinally into several tubules, we should have the condition found in *Caponia*. To illustrate this, as well as the arrangement and origin of the tubules on the anterior wall of the ante-chamber, I have given a series of consecutive transverse sections (text-figs. 8—15), of which text-fig. 8 through the ante-chamber represents the most posterior of the eight. It will be observed that the tubes are frequently

somewhat flattened dorso-ventrally at their origin, only to divide into a couple or more cylindrical tubes in the next section, e.g. the lowest tube in text-fig. 9. The tracheal tubes are not long, being only about three or four times the length of the ante-chamber, and they do not enter the abdominal pedicel. All, or nearly all, are several times branched, the branches becoming slenderer towards the apex where they frequently end in a short fork (fig. 10). They are either cylindrical or compressed, being then mostly flattened dorso-ventrally, like the saccules of lung-books, and are lined internally, except quite at the base, with a fine, probably spiral, thickening of the cuticle, just like the tracheal tubules of the *Dysderidæ*, etc. The bi-nucleate columns of the original saccules have, of course, disappeared. The anastomosing spines of the ante-chamber enter the bases of the tubes for a short distance and the free edges of the tubes bordering on the cavity of the ante-chamber have very much the appearance of those of pulmonary saccules in sagittal sections (cf. fig. 5 of the lung-book of *Harpactes* with figs. 11 and 12 of the tracheæ of *Caponia*). In a young specimen examined the tubules were much fewer than in any of the adult specimens, hence it is evident that they increase in number with the growth of the spider.

In addition to the above there is a small group of tubules which start from a slightly projecting portion of the ante-chamber at its base on the medial side. This group is composed of a bunch of four tubules, which may, however, subdivide into several more. They take at first a transverse medial direction and then bend and run some distance backwards on the lateral side of the second pair of tracheæ (fig. 9, *med. tub.*). This group of tubules has no equivalent in the lung-book of the *Dysderidæ* and is to be looked upon as a new formation. They may, perhaps, be the posterior group of six tubules represented in one of Bertkau's figures (Simon, fig. 294, or Lamy, fig. 25).

The second pair of tracheæ of *Caponia* forms perhaps the most complete and extensive tracheal system known

in any spider. It furnishes the abdomen, cephalothorax and appendages with a very great number of fine air-tubes, but only a portion of these are represented in fig. 9 in which the terminal parts of the numerous branching tubules, often measuring only 3μ in diameter, have not been drawn in.

The pedicel of the posterior tracheæ opens into a capacious tracheal trunk (p. 545, text-fig. 16, *c.tr.*) lined with spines, which runs forwards at an inclination of about 45° for a very short distance only before dividing into two sub-cylindrical branches of equal length and thickness, which may be called the cephalothoracic trunks (fig. 9, *ceph.tr.*), and run horizontally forwards into the cephalothorax, becoming thinner anteriorly. They remain in contact one above the other but the upper one a little more to the lateral side, and are somewhat flattened along the place of contact. Soon after passing through the pedicel of the abdomen they each break up into a bunch of fine tubules which then spread in various directions. Those of the right upper trunk are drawn in fig. 9, from which it will be seen that most of the tubules of the right trunk cross over to the left side and, generally remaining unbranched, enter the coxæ of the left appendages, whereas only the posterior appendages of the right side receive tubules from this trunk. Several of the tubules give off dendritic branches (*d.br.*), which ramify within the cephalothorax and supply its upper part with air. While the inferior cephalothoracic trunks remain unbranched except at apex, the two upper ones each give off a small branch (fig. 9, *br.*) from the upper side near the middle. This branch is arborescent in form and divides into a number of tubules which supply the region above the anterior pair of tracheæ.

In addition to the two anterior trunks each of the short main trunks also gives off from its posterior side at base a cylindrical posterior trunk of half the diameter of either of the anterior ones. This trunk, which may be called the abdominal trunk, runs first in an upward and medial direction, and then curves and runs horizontally towards the

posterior part of the body. It is of a pronounced arborescent form, but only its larger branches are provided with spines.

The spines which line the various trunks within are arranged in longitudinal rows (fig. 4, *spi.*), and are connected at apex by transverse threads (*thr.*), which, however, also anastomose with adjacent threads, the whole arrangement being very much as in *Dysdera* (see Lamy :02, pp. 180 and 181, figs. 20 and 21). The larger tracheal branches in the abdomen have similar spines, but the finer branches or tubules in the abdomen and all the tubules in the cephalothorax have the transverse threads only, but no spines.

Each of the short main trunks (text-fig. 16, *c. tr.*) is also produced laterally, together with the pedicel and spiracle, beyond the points of origin of the three principal branch trunks to form a small but very curious, lateral pocket (fig. 9, *l. p.*), indicated by Bertkau in his two figures. This pocket is divided into a higher posterior and a lower anterior compartment, each compressed from before and behind. The posterior compartment (fig. 8, *p. c.*) is provided with anastomosing spines, directly continuous with those of the main trunk, along its upper and medial edges only (*spi.*), the rest of its surface being spineless but much crumpled. It gives off three small branches, viz. one from the upper edge in an antero-lateral direction, and one each from the upper lateral and medial angles. These soon subdivide and end in fine tubules; they are shown in fig. 9, and have also been indicated in Bertkau's figures.

The anterior compartment (figs. 6—8, *a. c.*) of the lateral pocket is lined with anastomosing spines on its anterior side (fig. 8, *spi.*), but the upper part of this side and that of the posterior side is furnished with short sharp spines, the rest of the posterior surface being spineless but much crumpled. From the upper edge of the compartment two (in the male) or three (in the female) peculiar stout rods or processes (*rd.*) of the cuticula project downwards into the lumen of the compartment, each being armed at the base with some

minute, sharp, conical spines, and with some longer ones towards the apex.

The hypodermis of the upper and posterior sides of the anterior compartment, and especially that of the anterior side of the posterior compartment, is much thicker than elsewhere, and its cuticula has a corrugated appearance in sagittal sections, and stains more deeply than the adjacent cuticula does (fig. 8). Plainly the whole of this structure has some function other than respiratory. The hypodermis bearing the three rods is connected at base by means of a strand with some cells or fibrous tissue, which may be a nerve (fig. 8, *nr.*). As, however, the specimens were not especially preserved for histological purposes, it is impossible to say anything definite about the character of these structures, except that the rods certainly strongly resemble sense organs.

There are well-developed, transverse, intertracheal folds of the integument connecting the spiracles of each pair and already indicated by Bertkau in his figures. That of the posterior pair (fig. 9, *tr.fld.*) encloses a spinous canal of communication (p. 545, text-fig. 16, *can.*), which connects the lumen of the short main trunks (*c.tr.*) with one another. In the anterior segment there is no spinous canal of communication, although the lateral parts of the fold are directly continuous with the pedicels of the anterior pair of tracheæ.

The well-developed anterior (epigastric) fold (text-figs. 16 and 18, *ep.fld.*) is strongly inclined forwards or even horizontal, especially throughout the median half, where the genital duct opens into its anterior wall. Each lateral fourth of the fold, lying (in the female) between the opening of the genital duct and the tracheal pedicels, appears twice bent (text-fig. 18), first upwards or slightly backwards, and then more sharply forwards and downwards, the whole of the anterior deflected portion (*l.*) serving for the attachment of a broad and powerful muscle (No. 4 in text-figs. 16 and 18). Near to the trachea the upper part of the fold is somewhat inflated, and produced upwards to form a conspicuous enta-

pophysis (text-fig. 16 and fig. 8, *ec.t.* 8) for the attachment of the muscles 1—3. The hollow entapophysis of *Caponia* much resembles the solid one of the *Oonopidæ* (fig. 1), and muscle No. 4 in the former corresponds exactly as regards its place of attachment to the muscle 38 in the latter, although not homologous with it.

The muscular system of the abdomen of *Caponia* is in some respects very peculiar. That connected with the respiratory segments (with the exception of the muscles of the oviducts) is given in text-figs. 16—18 and explained in the following list:

*List of the Muscles in Text-figs. 16—18.*¹

1 and 2. From the entapophysis *ec.t.* 8 to upper and middle part of side of abdominal pedicel.

3. Longitudinal from the entapophysis *ec.t.* 8 to the cephalothorax.

4. From lower lobe of the entapophysis *ec.t.* 8 to ventral integument of anterior respiratory segment.

5. From upper part of side of abdominal pedicel to ventral integument of anterior respiratory segment. (1 and 5 are inserted together anteriorly.)

6. Dorso-ventral on side of pedicel. (2 and 6 are attached to the same ectodermal infolding on the medial side of 5.)

7. Oblique dorso-ventral from hinder end of the ectodermal tendon *ec.t.* to the ventral fold *fd.* 1 of abdominal pedicel.

8. Oblique dorso-ventral from hinder end of the ectodermal tendon *ec.t.* to the integumental fold *fd.* 2 on lateral side of posterior spiracles.

9. From the ectodermal tendon *ec.t.* to anterior integument of abdomen.

10. Two muscles from the ectodermal tendon *ec.t.* to dorsal integument of abdomen.

11. Short muscle from lateral part of posterior (upper) wall

¹ Throughout this paper homologous muscles are indicated by the same numbers.

of anterior tracheal ante-chamber in a dorso-lateral direction to the body integument on the lateral side.

12. Longitudinal parietal along ventral integument of abdomen.

13. From posterior intertracheal fold to integument of abdomen (many or most of the strands continuous with 12).

23*a*. Longitudinal from *x* to the spinners, running alongside of the abdominal tracheal trunk for the greater part of its course and dividing posteriorly into several muscles, the lowest of which is attached to the lateral or middle part of the anterior side of the mesial anterior spinner (thus apparently corresponding to 27 or 28 in *Segestria*).

30. From the apex of the entapophysis *ec.t.* 8 in a dorso-lateral direction to integument of abdomen on lateral side of anterior spiracles (inserted immediately behind 11).

30*a*. Longitudinal from the apex of the entapophysis *ec.t.* 8 to *x*. (See also Pl. 28, fig. 8, *m.* 30 *a*.)

32. From *x* to posterior side of posterior intertracheal fold (inserted at extreme medial end of the spiracle).

33. From *x* in an antero-dorsal direction to the oviduct, meeting the latter at the point where the muscle ends in the figure. (The muscles 23*a*, 30*a*, 32, and 33 are continuous with each other at *x* on the lateral side of the abdominal tracheal trunk.)

34. Oblique from medial and posterior side of the common basal part (*c.tr.*) of tracheal trunk to anterior intertracheal fold (inserted on medial side of the entapophysis *ec.t.* 8).

35. From upper edge of posterior intertracheal fold to base of anterior intertracheal fold (many strands apparently continuous with 13).

36. Subtransverse from posterior medial edge of anterior tracheal pedicel (in a transverse line with the cross \times in text-fig. 17) to between the upper and lower cephalothoracic trunks of the posterior trachea. (See also fig. 8, *m.* 36.)

37. From the spineless, basal, posterior part of the second trachea and its lateral pocket in a dorso-lateral direction to the integument on the lateral side of the second spiracle

(inserted next to 8, and just behind but slightly lower than 30). (See also fig. 8, *m.* 37.)

The most remarkable features of this muscular system are the complete absence of all entochondrites, and as well as of those segments of the great, ventral, longitudinal muscles which belong to the second respiratory and the anterior spinner segments (somites 9 and 10).

The muscles 1—10 are identical in the *Dysderidæ* and *Caponiidæ*, 1—4, however, being attached in the latter directly to the anterior side of the entapophysis (*ec.t.* 8) without the interposition of an entochondrite. No. 4 is a very broad and powerful muscle, being attached, as already explained, to the whole anterior side of the lateral deflected lobe (*l.*) of the epigastric fold, and is represented in the *Dysderidæ* by several feeble strands only. The muscles 1—3 are attached in *Caponia* to the prominent lateral entapophysis (*ec.t.* 8), which is, therefore, plainly homologous with the entapophysis of the pulmonary segment of the *Dysderidæ* (p. 530, text-fig. 6, *ec.t.* 8) and other dipneumonous spiders. It may be noticed that in *Caponia* this entapophysis has completely taken the place of the entochondrite (*t.* 8) of the *Dysderidæ*, its anterior surface being correspondingly expanded to take the four large muscles.

The three muscles, 8, 11, and 30, which in the *Dysderidæ* are attached to the entochondrite *t.* (p. 530, text-fig. 7), are also represented in *Caponia*, only here the lateral ends of these muscles are attached separately to the body integument, and are more dispersed (although still quite close together) owing to the absence of the entochondrite, and 11 runs parallel to the transverse plane, while in *Segestria* its fibres lie in sagittal planes.

The parietal muscle 12 is a part of the abdominal muscular sac which lies immediately within the outer hypodermis and envelops the intestines. The ventral strands of the sac are here longitudinal, and form a continuous layer from side to side, where they are again continuous with the lateral walls of the sac. Anteriorly the ventral strands of the sac ascend

(No. 13) to the upper edge of the posterior intertracheal fold along its whole extent, but the descending strands (No. 35) on the anterior side of this fold are only met with in the lateral part, being absent from the median part of the fold. A similar parietal muscle is met with in the *Dysderidæ* and *Oonopidæ*, differing only in so far that the lateral ascending strands (No. 13, p. 530, text-fig. 6) are attached to the large entochondrite (*t. 9*) of the tracheal segment.¹

I could find no trace of the usual medial longitudinal muscles corresponding to 15 and 18 in the *Dysderidæ*, and connecting the anterior entapophysis (*ec.t. 8*) with the spinners on the medial side of the tracheæ. In fact, the only muscle connecting the respiratory segments with the spinners, and lying inside of the abdominal muscular sac, is the slender muscle 23*a*, which, however, lies on the lateral side of the trachea, and is, I think, probably homologous with muscle 23 of the *Dysderidæ*. This little muscle in *Caponia* divides posteriorly into at least two muscles and the most ventral of these branches,² corresponding to 27 or 28 of *Segestria*, is attached to the lateral (♂) or middle (♀) part of the anterior side of the mesial anterior spinners, which I take to represent the anterior pair of the *Dysderidæ*. Anteriorly 23*a* unites with three other small muscles, 30*a*, 32, and 33, at a point *x* at the base of the lateral side of the abdominal tracheal trunk (*abd.tr.*). The four muscles are here in contact with the trachea, and their fibres intermingle without forming an entochondrite. One of them, 30*a*, passes on to the apex of the entapophysis (*ec.t. 8*), and

¹ The abdominal muscular sac in other spiders has been described by various authors, particularly by Causard ('96, pp. 22-24, pl. iii, figs. 1 and 2), and more recently by Lamy (:02, p. 158, pl. vii). Of the muscular nature of its fibres there can be no doubt whatever, as the typical transverse striations may be frequently observed in the *Caponiidæ*, the *Dysderidæ*, and other forms.

² I could not make out where the posterior ends of the dorsal branch or branches were attached.

may possibly represent a strand which became separated from 30 when the lateral entochondrite (p. 530, text-fig. 7, *t.*) disappeared.

From a comparison with the *Dysderidæ* it is evident that the tracheæ of the second respiratory segment in *Caponia* correspond to those of the *Dysderidæ*, and, like the latter, are to be considered as entirely homologous with lung-books, for there is no evidence that entapophyses took any part in their formation.

The receptacula seminis are paired, and consist of an enormous dilation of each oviduct apparently at the point where the ectodermal and mesodermal elements of the duct meet. They are placed nearer the upper part of the abdomen just above the area enclosed between the four spiracles, but extend for some distance to the front and behind this area as well. The ventral wall of each dilation has a cuticular lining, but the dorsal wall and the greater part of the side walls have none. Apparently the latter represent the mesodermal part of the oviduct and the former the ectodermal part. The ventral wall with its cuticula is continuous with the basal portions of the oviducts, which open into the lateral ends of the unpaired median portion of the duct. This latter again opens into the epigastric fold along a wide cleft occupying about one half of the distance between the two anterior tracheæ. The spacious lumen of each receptaculum contains coagulated stainable matter and numerous spermatophors. This form of receptacula is apparently quite unique amongst spiders, the usual ones which open directly into the epigastric fold, and are evidently invaginations of the body integument, being quite absent in *Caponia*.

GENERAL CONCLUSIONS.

A tracheal system may be imagined to have been evolved out of a lung-book in either of the following ways:

- (1) The pulmonary saccules may have been converted into

more or less cylindrical tubes (say, by longitudinal division), accompanied by the disappearance of the bicellular columns of the septa. The trachea thus produced would be composed of an ante-chamber formed out of the pulmonary ante-chamber, with a bunch of tubules on its anterior surface, formed out of metamorphosed pulmonary saccules. This metamorphosis does not involve a reduction in the effectiveness of the respiratory organ, and I imagine the trachea so produced to be in no way inferior, but rather superior, to the lung-book. Accordingly it would doubtless increase in size and take over the main respiratory functions, and this would be accompanied, in the case of the second respiratory segment, by a corresponding reduction in the number of the leaves of the anterior lung-books, in accordance with Lamy's law of the inverse correlation between the size of the tracheæ and the number of the lung-leaves.

(2) The saccules may have disappeared, leaving only the pulmonary sac or ante-chamber, which would then constitute a trachea, and may subsequently elongate or even acquire secondary branches. These latter, however, would not be homologous with pulmonary saccules. This method of origin really consists in a reduction in the effectiveness of the respiratory organ of the somite, and would be accompanied, in the case of the second respiratory segment, by a corresponding increase in the number of the leaves of the anterior lung-books, which would then become the principal organ of respiration. This, then, would be exactly the opposite process to that which would have taken place in the first case.

It appears to me very probable that both these methods of origin have actually occurred in the *Araneæ*, the first method being applicable to the *Dysderidæ* and their allies, and the second to the rest of the tracheate spiders. It will be convenient to take these two sections of spiders in turn.

(1) The *Dysderidæ*, *Oonopidæ*, and *Caponiidæ*, being those forms with the tracheal spiracles far apart and not moved backwards, i. e. still nearly in their primitive positions.—If, after the metamor-

phosis of the saccules into the tubules in the case of the first of the two methods given above, we further imagined the ventral part of the ante-chamber to lengthen slightly, we should have almost exactly the condition found in the anterior pair of tracheæ of *Caponia*, which differs from this ideal case only in two minor points, viz. in the presence of transverse or spiral thickenings in the tubules instead of small spines, and of a medial group of tubules at the base of the ante-chamber. In fact, as I have shown above, the anterior pair of tracheæ of *Caponia* may be taken to represent the most primitive form of metamorphosed lung-books known in which the saccules still persist as tubules.

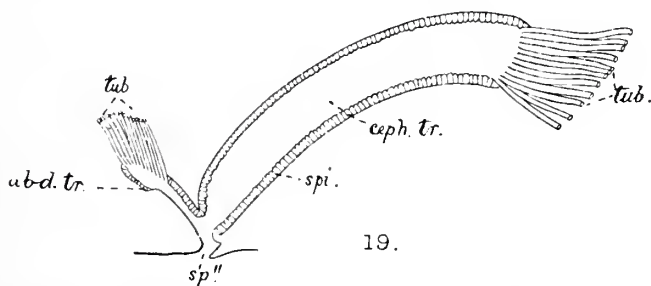


Diagram of a trachea of *Harpactes Hombergi* (ad. ♂), seen in section. Magn. 134. *abd. tr.* Abdominal branch of the trachea. *ceph. tr.* Cephalothoracic trunk. *sp''*. Spiracle. *spi.* Anastomosing spines. *tub.* Tubules.

The second pair of tracheæ in *Caponia*, being wholly homologous with lung-books, plainly belong to the same type as the tracheæ of the *Dysderidæ* and *Oonopidæ*, and are merely somewhat more complicated by the duplication of each of the cephalothoracic trunks and the elongation of the abdominal branch of the latter. The simplest form of this type, such as that found in *Harpactes* (text-fig. 19) and in *Calculus* (in both of which the anastomosing spines of the trunks still form a simple network and do not bear a spiral thread or inner perforated tube), may be easily derived from the anterior tracheæ of *Caponia* by merely exaggerating the tubular elongation of the ante-chamber, already commenced

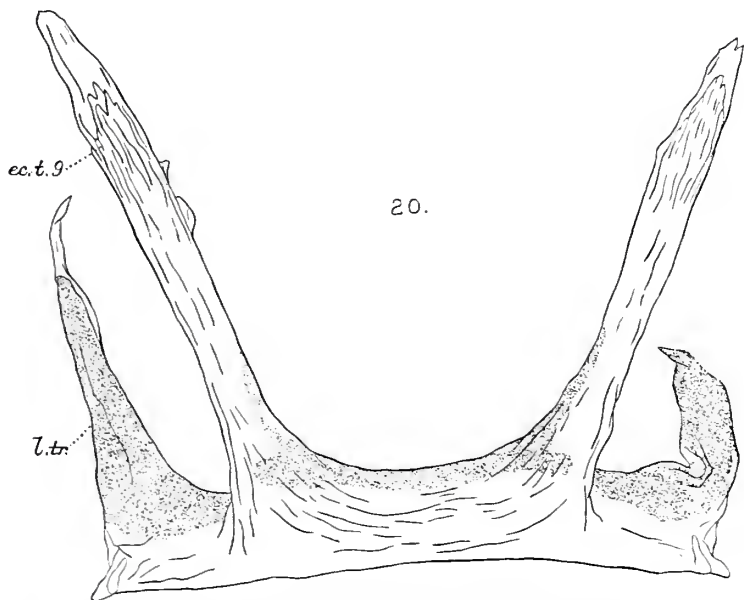
in the anterior tracheæ, and by the addition of the short posterior branch in place of the medial basal group of tubules. In such case the bunch of tubules at the anterior end of the cephalothoracic trunks (*ceph.tr.*) would represent metamorphosed pulmonary sacculles, but those of the posterior abdominal branch (*abd.tr.*) would be, of course, new formations.

In a previous paper (:09) I had already indicated the possibility of the anterior bunch of tubules being derived from sacculles, but after studying the tracheæ of *Caponia* more thoroughly, I am now much more strongly inclined to believe that such has actually been their origin. A study of the embryology would, however, be necessary to settle this interesting point.

If, as I have assumed, the posterior pair of tracheæ in *Caponia* and those in the *Dysderidæ* had a common origin, it follows that the anterior pair of tracheæ in the former must have developed later and independently of the posterior pair, and that, therefore, tracheæ must have originated from lung-books at least twice in the *Araneæ*. The same conclusion would follow even if we assumed that both pairs of tracheæ in *Caponia* originated at the same time and not as separate metamorphoses, for in that case both pairs of tracheæ must have originated independently of those of other tracheate spiders, since these latter still possess the anterior pair of lung-books.

The morphology of the respiratory segments bears out the view that the three families discussed above are intermediate in position between the mygalomorphous spiders and the rest of the arachnomorphous forms. This view was demonstrated by Bertkau ('78) a good while ago for the *Dysderidæ* and the *Oonopidæ*, and this author even went so far as to include these families with the mygalomorphous forms in a common group, the *Tetrasticta* (i. e. with four stigmata). No doubt these two families are the most primitive of the three, but the *Caponiidæ* may be considered as an allied but in several respects a very aberrant type, standing apart from the other two families.

(2) Forms with the tracheal spiracles approximated and moved more or less toward the hinder end of the body.—All the remaining tracheate spiders come under this heading,¹ and may be considered in two groups, viz. group A, those in which the entapophyses of the tracheal system are non-respiratory (Filistatidæ, Sicariidæ, and Palpimanidæ), and, group B, those in



Tracheal apparatus of *Filistata capitata* (after Lamy). *ec.t.9.* Non-respiratory entapophysis. *l.tr.* Lateral or tracheal sac. Magn. 100.

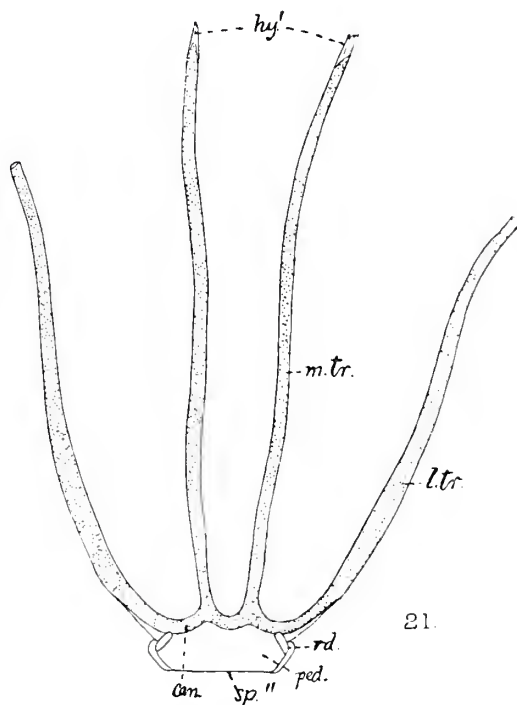
which these entapophyses have been transformed into tracheæ (including all the remaining families).

A very simple and interesting type of tracheæ of the first group is that of *Filistata* (text-fig. 20), which is known to us from Lamy's description.

The simplest and most usual type of the second group, a

¹ In some cases, e.g. *Argyroneta*, the common tracheal spiracle appears to have secondarily moved forward again.

type found, according to Lamy, in about half of the total number of genera examined, consists of four simple tracheal trunks united at base, as in text-fig. 21. It is known from the embryology that the lateral trunks of this type were derived from the pulmonary sac or ante-chamber of a lung-book, and are, therefore, homologous with the lateral trunks



Tracheal apparatus of *Linyphia triangularis*. Cl. (ad. ♀. caustic potash). Magn. 80. *can.* Canal of communication between the tracheal trunks. *hy'*. Terminal chitinous fibres by which the medial or tendinal trunks (*m. tr.*) are attached to the entochondrites. *l. tr.* Basal portion of lateral trunk. *ped.* Pedicel of trachea. *rd.* Lateral supporting rod. *sp''*. Spiracle.

in Filistata,—while the medial trunks represent metamorphosed entapophyses (ectodermal tendons of muscles), and are, as Lamy (p. 172) has pointed out, homologous with

the medial trunks of *Filistata*, which this author has shown to be entapophyses.

In both groups more complicated types than the two simple ones just described are frequently found, and it is important to notice that this complication takes place along different lines in each group. Thus in group A some *Sicariidæ* and *Palpimanidæ* were found by Lamy (p. 176, fig. 15, and p. 188, fig. 30) to possess branched tracheæ, the branching being confined to the lateral or tracheal trunks. In group B, on the other hand, in all cases where the lateral and medial trunks can be identified from Lamy's figures and show different degrees of development, it is invariably the medial trunks which show the greatest complexity and the highest degree of development as respiratory organs.¹ This rule appears to me to furnish the key to the phylogeny of the tracheæ in these spiders. We may also fairly deduce from it that the medial tracheæ must be more efficient as respiratory organs than the lateral tracheæ are, and the reason for this, as I have already pointed out (:09), may be their position in the large ventral sinus containing venous

¹ This is self-evident from Lamy's excellent figures in many cases e.g. *Œcobiidæ* (Lamy, p. 170, fig. 10), *Argiopidæ* (pp. 197—199, figs. 38—42), *Thomisidæ* (pp. 206 and 207, figs. 49 and 50), and *Agelenidæ* (pp. 214—216, figs. 59—61). In arborescent types of tracheæ (see my paper, :09) it is not so self-evident, but the same conclusion may be deduced from the great similarity which this form of trachea shows to that of the *Attidæ*, of which the identity of the parts is known from the embryology. There remain, however, certain *Dictynidæ* and *Agelenidæ*, the homology of whose tracheæ cannot be ascertained with any degree of certainty from Lamy's figures. In *Argyroneta* I found (:09), from the position of the muscles and entochondrites, that the entire trachea appears to have been derived from the medial trunks, but I have had no opportunity of examining any of the other forms, viz. *Dictyna* (Lamy, p. 169, fig. 8), *Antistea* (p. 213, fig. 57), *Cybæus* (p. 217, fig. 62), and *Chorizomma* (p. 219, fig. 64). If these, too, could be proved to follow the rule given above, the arguments in the following pages would be greatly strengthened. I may add here that in the marine *Agelenid*, *Desis tubicola*, Poc., the tracheæ, which have not been hitherto described, closely resemble those of *Attus*.

blood. This greater efficiency would account for the higher degree of development of the medial trunks in many forms.

Now out of the twenty-four families in which the medial entapophyses have been converted into tracheæ, twenty-two, according to Lamy's investigations, possess tracheal systems consisting of four simple tubes (p. 554, text-fig. 21) in some of their genera at least, while eight of these families possess both this simple type and more complicated types as well. In fact, only two very small families (*Ecobiidæ* and *Prodidomidæ*) have the more complicated type only. And since the type with branched medial trunks must have been derived from the type with simple trunks, as the medial ones were originally simple entapophyses, we may fairly conclude that the common type with four simple tubes is the primitive one for the entire group, and that the more complicated types must have been developed from the simpler types within each family separately and independently of similar complicated types in other families. This statement is in agreement with Lamy's view referred to in the introduction, except that this author does not consider any particular type as more primitive than another.

Again, it is evident that the type of trachea in which the entapophyses are not respiratory must be considered as more primitive than those in which they are respiratory, since the more efficient medial tracheal trunks would not be likely to revert to their original function after once being metamorphosed. Hence the tracheæ of the *Filistatidæ*, *Sicariidæ*, and *Palpimanidæ* must be looked upon as more primitive than those of group B with metamorphosed medial trunks, and it seems to me very probable that the tracheæ of the latter group were originally derived from some such form as that found in *Filistata* (p. 553, text-fig. 20). In this spider the tracheæ are placed, according to Lamy (:02, p. 172, fig. 11), about midway between the spinners and the inter-pulmonary fold. The anterior end of each of the tracheal entapophyses is situated near this fold, and consequently the segments of the longitudinal muscles between the entochon-

drites of the pulmonary and tracheal segments are doubtless quite short. If now the tracheal spiracle moved to the hinder end of the body and the entapophyses elongated correspondingly and became converted into a trachea we should get the type represented in text-fig. 21 (p. 554), which I consider to be the primitive type of all forms with metamorphosed entapophyses. The anterior ends of the entapophyses would still be near the interpulmonary fold, and the connecting muscular segment would still be quite short, as it always is in the spiders of group B.

In *Scytodes* and *Palpimanus* the spiracle has moved to the hinder part of the body without any additional lengthening of the entapophysis. Hence in these two forms the segments of the longitudinal muscles belonging to the tracheal somite are greatly elongated, and in this respect these forms (and allied genera) are apparently unique.

The *Pholcidae*, which Bertkau found to have no tracheæ at all, were perhaps derived from some form with a type of trachea similar to that of *Filistata*, since according to Lamy's investigations a pair of entapophyses persists in some *Pholcidae* in the same position in which those of *Filistata* are found (:02, pp. 191 and 192, figs. 32 and 33). The *Pholcidae*, therefore, should perhaps belong, as regards the structure of their ninth somite (the tracheal segment in other spiders), to the same group as the *Filistatidae*, *Sicariidae*, and *Palpimanidae*.

In a previous paragraph two possible solutions were suggested for the derivation of a tracheal system from lung-books, one of which appeared particularly applicable to the tracheal system of the *Dysderidae*, etc. Now the second method suggested, which consists in the reduction of the respiratory functions of the lung-books by the abortion of the sacculæ, appears to me to exactly meet the conditions found in the spiders with four simple tracheal trunks (or with two tracheal trunks and two entapophyses), in which the lung-books have numerous leaves, and obviously play the most important part in the respiration. The size of the

lung-books in *Filistata* is not known, but judging from Lamy's figure (:02, p. 172, fig. 11) they appear here, too, to be very large to compensate for the feeble development of the tracheæ. This relatively greater size of the anterior lung-books is exactly what I have explained should take place if the posterior lung-books became reduced to their ante-chambers only.

If we imagined a tetrapneumonous spider with both pairs of lung-books connected by interpulmonary folds (the arachnomorphous spider *Hypochilus* appears to be such a form), and the entapophyses prominently developed in the second respiratory segment, as well as in the first, it would be perfectly simple to derive from it a form with tracheæ exactly resembling those of *Filistata*. All that would be necessary would be that the saccules of the second pair of lung-books should disappear, leaving the two ante-chambers only; and that the two spiracles should come a little nearer together so as to form practically one opening with the intertracheal fold. It appears to me very probable that the tracheæ of *Filistata* and of all other spiders (except the *Dysderidæ* and their allies) had this mode of origin, which is in entire agreement with the account given by Lamy of the structure of the tracheæ in *Filistata*. The two short lateral tracheal sacs (p. 553, text-fig. 20, *l.tr.*) of this form are lined with spines and triangular in shape, exactly resembling a pulmonary sac deprived of its saccules. A study of the *Hypochilidæ* would probably throw some further light upon this subject, since here the second pair of lung-books are placed, according to Simon's figure ('Hist. Araign.,' 2nd ed., i, p. 201, fig. 145), far back, about midway between the anterior pair and the spinners, corresponding exactly in position to the tracheal system of the *Filistatidæ*.

I have made no attempt to explain the origin of those tracheal tubules, which cannot by any line of argument be derived from pulmonary saccules. The numerous tubules emitted from the large tracheal trunks in the *Attidæ* are a case in point, since these trunks, with the exception of their

lateral basal lobes, are metamorphosed entapophyses. These tubules may have originated simply as outgrowths of the trunks, and would then, of course, be of ectodermal origin. Ray Lankester is of opinion (:04, p. 223) that the tracheal tubules in *Arachnida* (and in all other *Tracheata*) have developed "by adaptation of the vasifactive tissue of the blood-vessels," which have come to open in the case of the *Arachnids* into the lung-chambers (and elsewhere). Instances of mesodermal tubes attaching themselves to, and opening into ectodermal invaginations are, of course, well known, e. g. the genital ducts. No actual embryological observations, however, exist, so far as I am aware, regarding the development of the fine tracheal tubules in *Arachnida*. In *Attus floricola* no trace of these tubules was found up to the stage formed at the second moult, and I had no later stages at my disposal.¹

Summary.—The theoretical suggestions in the preceding paragraphs may be summed up as follows :

In the first place I suppose the sacculæ of the second pair of lung-books to have been converted into tracheal tubules in the common ancestor of the *Dysderidæ*, *Oonopidæ*, and *Caponiidæ*. The resultant tracheæ then increased in size, and, as the number of the leaves of the anterior lung-books decreased in inverse ratio, the former became the principal organs of respiration. The second pair of spiracles retained their position, or may even have moved slightly forwards, and the conversion of the entapophyses into tracheæ could not take place here, and would, moreover, be quite unnecessary. In the *Caponiidæ* the anterior pair of lung-books were converted into tracheæ in a similar manner, but at a later period, and independently of the conversion of the posterior pair; but as the latter already provided almost the

¹ A paper by R. Janeck entitled "Entwicklung der Blättertracheen und der Tracheen bei den Spinnen" has recently appeared (*Jena Zeitschr. Naturw.* xlv, Hft. 2—4, 1909), but I have not hitherto had access to this publication.

entire body with tracheæ, the anterior pair did not further increase in size.

In the second place, in the progenitor (or progenitors) of the remaining tracheate spiders, the posterior lung-books became reduced in size and effectiveness by the disappearance of their saccules, accompanied by an increase in the number of the leaves of the anterior lung-books. Further, the posterior spiracles became approximated and united to a single spiracle, and moved towards the hinder end of the body, thereby causing the entapophyses of the tracheal segment to elongate. In this condition the *Filistatidæ*, *Sicariidæ*, and *Palpimanidæ* have remained, with slight modifications, such as the division of the tracheal antechambers into branches in some forms. In the great majority of the families, however, the elongated entapophyses became transformed into a pair of medial tracheal trunks, thus producing a tracheal system consisting of four simple unbranched trunks, which is still found in some genera at least, in nearly all the families. A new factor having been introduced, viz. the presence of the respiratory entapophyses lying in the large ventral sinus containing venous blood requiring aëration, we accordingly find the second respiratory segment again taking a prominent part in the respiration in many forms, owing to the increase in size and the branching of the medial trunks, accompanied ultimately by a corresponding reduction in the size of the anterior lung-books, e. g. in the *Attidæ*. This method of origin of the tracheæ is independent of that of the *Dysderidæ* and its allies, and the tracheal tubules, when present, would here not be derived from saccules, but be new formations.

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EXPLANATION OF PLATE 28,

Illustrating Mr. W. F. Purcell's paper on "The Phylogeny of the Tracheæ in Araneæ."

ABBREVIATIONS.

a. c. Anterior compartment of lateral pocket of trachea. *a. tr.* Anterior trachea. *abd. tr.* Abdominal trunk of trachea. *ant.* Anterior side. *bd. c.* Blood corpuscles. *br.* Branch of trachea. *can.* Canal of communication between the tracheal trunks. *ceph. tr.* Cephalothoracic trunks of trachea. *cu.* Cuticula. *d. br.* Dendritic tracheal branches.

dors. Dorsal side. *ec. t. 8.* Ectodermal tendon (entapophysis) of the anterior respiratory segment. *ep. fd.* Epigastric fold (along hinder margin of anterior respiratory segment). *fd.* Fold in dorsal wall of tracheal ante-chamber, to the lateral part of which the muscle 11 (text-fig. 17) is attached. *g. o.* Opening of the genital duct into the epigastric fold. *hy.* Hypodermis. *k.* Median keel on ventral side of receptaculum seminis. *l. p.* Lateral pocket of trachea. *lat.* Lateral side. *m.* 1-37. Muscles (see numbered list in text). *m.* 38. Obliquely transverse muscle from the anterior surface of the entapophysis. *ec. t. 8* to the sides of the keel of the receptaculum seminis. *m.* 39. Short muscle from the entochondrite *t. 8* in a medio-ventral direction to posterior side of epigastric fold. *m.* 40. Median muscle from ventral side of receptaculum seminis to ventral integument of body, running along posterior ventral edge of the keel of the receptaculum. *med.* Medial side. *med. tub.* Medial bunch of four tubules at base of anterior trachea. *nr.* Nerve? *p. c.* Posterior compartment of lateral pocket of trachea. *ped.* Pedicel. *post.* Posterior side. *pulm. a.* Pulmonary ante-chamber. *r. s.* Receptaculum seminis. *rd.* Sensory rods in trachea. *sept.* Septa (lamellæ) of lung-book. *sp'.* Spiracle of anterior respiratory segment. *sp''.* Spiracle of posterior respiratory segment. *spi.* Anastomosing spines. *spi'.* Points at which the tracheal spines are attached. *spm.* Spermatophors. *t. 8.* Entochondrite at posterior end of the segment of the ventral longitudinal muscle of first respiratory somite. *thr.* Transverse anastomosing threads borne by the tracheal spines. *tr. a.* Tracheal ante-chamber. *tr. fd.* Intertracheal fold along hinder margin of second respiratory segment. *tub.* Tracheal tubules. *vent.* Ventral side.

All the figures, except fig. 9, were drawn with the aid of a drawing apparatus. Transverse and sagittal sections are so arranged that the horizontal plane of the body is parallel to the lower edge of the paper.

FIG. 1.—(Zeiss, objective C, ocular II, spirits.) *Calculus bicolor*, adult ♀. Sagittal section through the entapophysis of the pulmonary segment.

FIG. 2.—(Zeiss, C. II, spirits.) Median section through the receptaculum seminis and epigastric fold, from the same series as fig. 1.

FIG. 3.—(Zeiss, C. II, warm Flemming's solution + alcohol.) *Harpactes Hombergi*, ad. ♀. Similar section to fig. 2.

FIG. 4.—(Zeiss, $\frac{1}{12}$ oil immers., IV.) *Caponia spiralis*. Internal chitinous threads of cephalothoracic tracheal trunks.

FIG. 5.—(Zeiss, C. IV, warm Flemming's solution + alcohol.) *Harpactes Hombergi*, ad. ♀. Sagittal section through a lung-book.

FIG. 6.—(Zeiss, A. IV, spirits.) *Caponia spiralifera*, ad. ♀. Transverse section through the basal part of the trachea of the second respiratory segment.

FIG. 7.—(Zeiss, C. IV, spirits.) Lateral part of fig. 6.

FIG. 8.—(Zeiss, C. IV, spirits.) *Caponia spiralifera*, ad. ♀. Sagittal section through the entapophysis of the first and the lateral tracheal pocket of the second respiratory segments.

FIG. 9.—(Caustic potash.) *Caponia spiralifera*, ad. ♀, showing tracheal system (the ends of the tubules of the tracheæ of the second respiratory segment are not drawn in).

FIG. 10.—(Zeiss, A. IV, caustic potash.) *Caponia spiralifera*, ad. ♀. Right anterior trachea from the medial side.

FIGS. 11 and 12 (Zeiss, C. IV, spirits.) Same series as fig. 8. Sagittal sections through the left and right anterior tracheæ respectively. In fig. 12 the cuticula of the posterior side and the hypodermis of both sides of the pedicel have not been drawn in.

Errata to Mr. W. F. Purcell's Paper, "Development and Origin of the Respiratory Organs in Araneæ."

Published in the Quart. Journ. Micr. Sci., vol. 54, Part 1,
September, 1909.

Page 2, line nineteen from top, for Scytodidæ read Sicariidæ.

Page 26, line five from top, for pp. 17-20 read p. 24.

Page 43, line twenty-one from top, for 49 read 35.

Page 54, line seven from top, for 20 read 46.

Page 59, line three from top, for left read right.

Page 71, line nine from top, for Scytodidæ read Sicariidæ.

Page 79, line four from bottom, for 33 read 40.

Page 82, line sixteen from bottom, for 17-44 read 25-28.

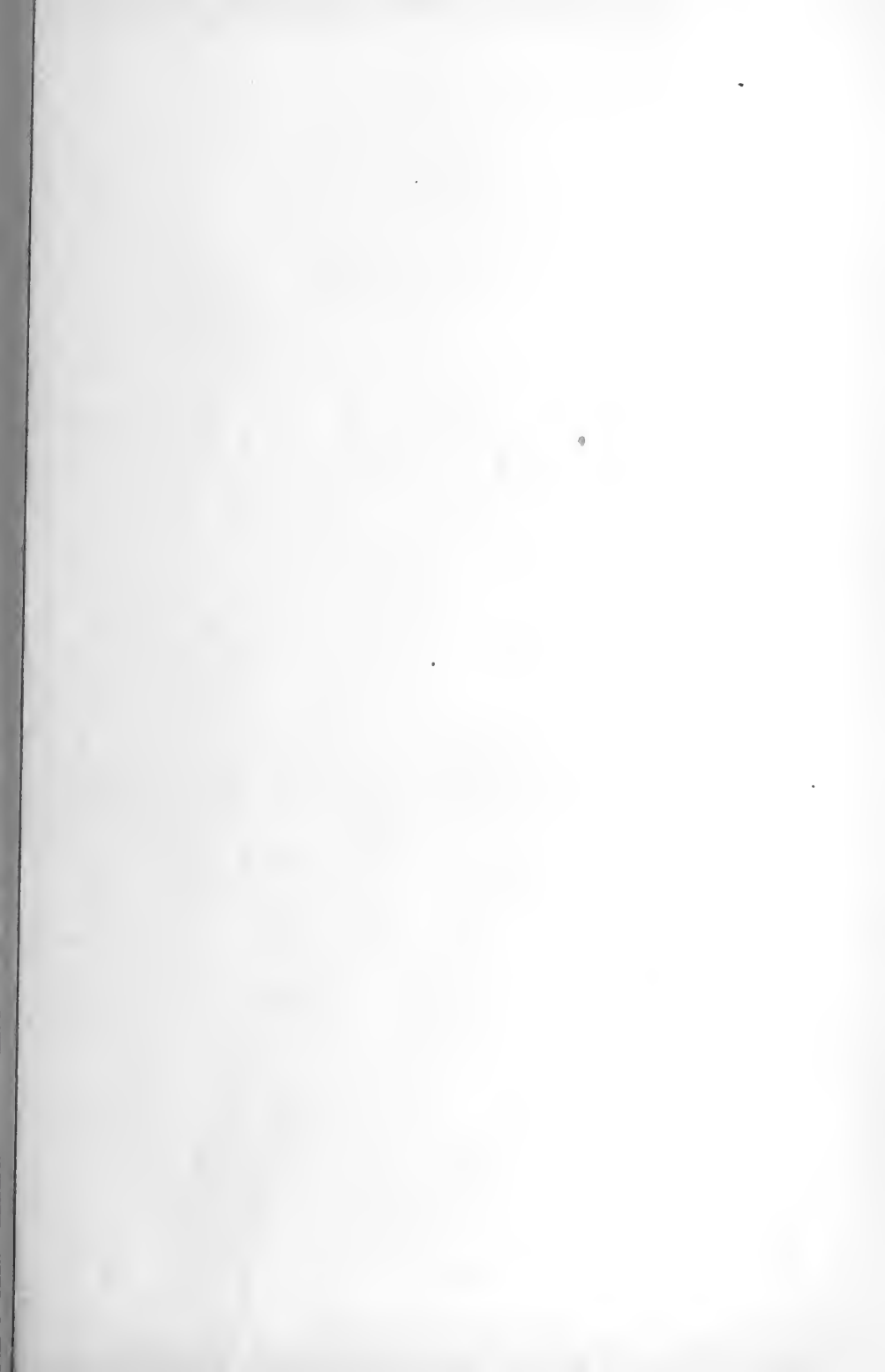
Page 103, line nineteen from bottom, for yellow read grey.

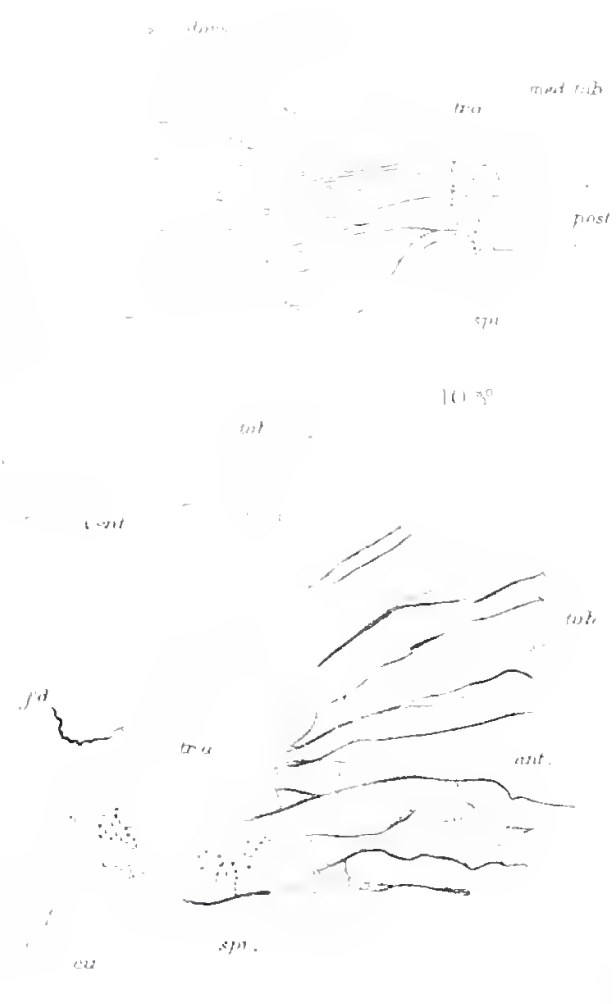
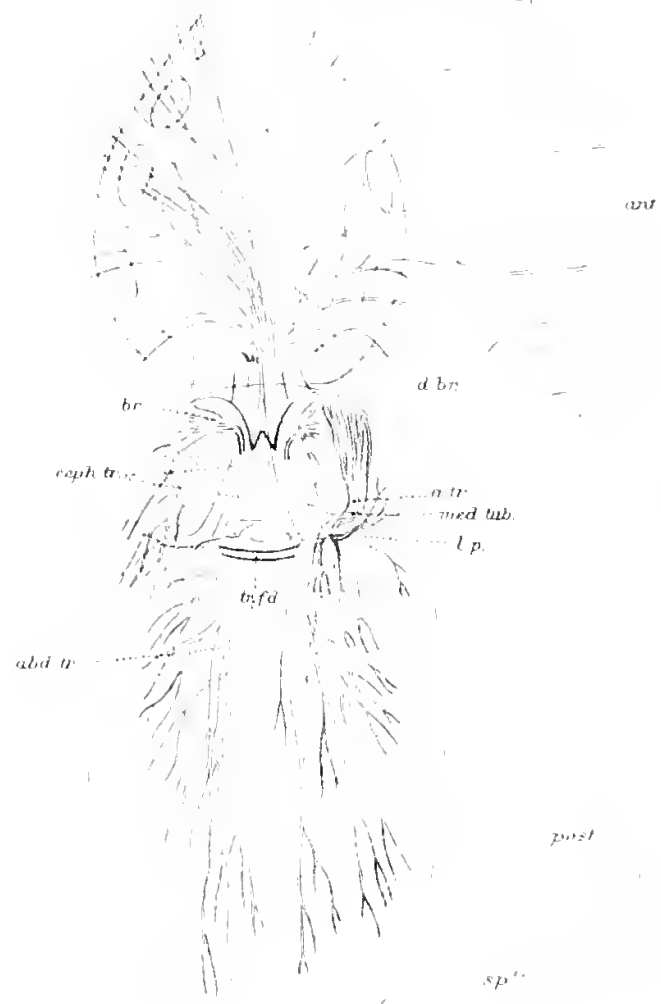
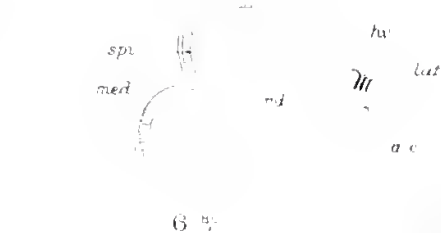
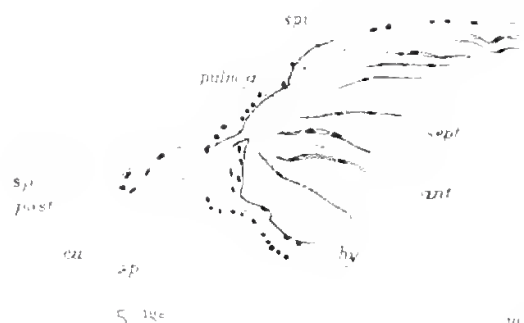
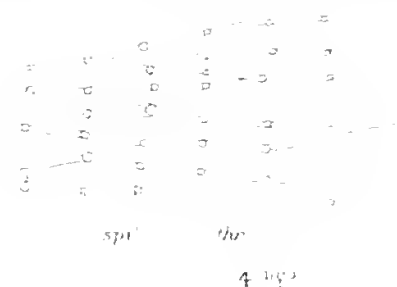
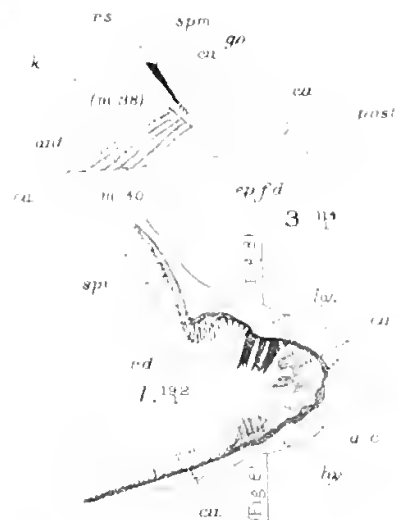
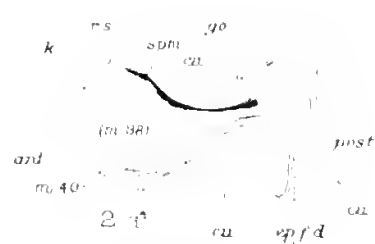
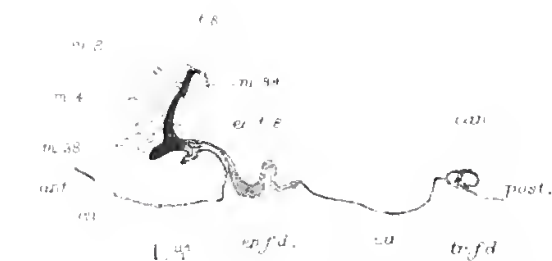
Page 105, line three from top, for anterior and posterior read ventral and dorsal.

Page 106, line thirteen from bottom, for anterior read posterior.

Page 108, line two from top, for 22A read 23A. Line eleven from top, strike out Adult or sub-adult spiders.

Plate I, figs. 6 and 6A, for *ab. app.* 8-11 read *ab. app.* 1-4.





11 1st

12 1st



On the Reproduction of *Kalpidorhynchus arenicolæ* (Cnghm.).

By
Margaret Robinson,
University College, London.

With Plate 29.

INTRODUCTION.

IN 1907 Mr. Cunningham described and gave a life-history of this gregarine in the 'Archiv für Protistenkunde.' The parasite was first noticed by Mr. De Morgan while dissecting some specimens of *Arenicola ecaudata* in this laboratory. Owing to pressure of other work Mr. Cunningham was unable to give a complete account of the reproduction, and he therefore suggested that I should, at some future time, try to find the first division nucleus with a view to ascertaining where the chromatin of its chromosomes came from. The latter half of this problem still remains unsolved; but after cutting many cysts into sections I did find the first spindle in a very early state, and I have been able to make one or two other observations which may prove to be not without interest.

METHODS.

The cysts were fixed with various fluids, Hermann's, Flemming's, corrosive sublimate and acetic acid, with and without the addition of formaline, Bonin's picro-formol and Brasil's picro-formol. The best results were obtained by using the picro-formol mixtures. The sections were stained

by Heidenhain's method. Following the directions given by Brasil (1905), I left them for twenty-four hours in the mordant, and for thirty-six or more in the hæmatoxylin. As plasma stains after Heidenhain I used a mixture of Licht-Grün and picric acid in equal parts dissolved in absolute alcohol, orange G., and eosin.

Two other stains of which I made use were Delafield's hæmatoxylin and that of Kleinenberg.

THE FIRST SPINDLE.

The nuclear membrane disappears gradually bit by bit, and the nucleus becomes more or less diffuse, assuming an irregular outline in the parts no longer contained by the membrane. Close to this uneven edge there appears a little cluster of rod-like pieces of very darkly staining chromatin. These small rods are connected together by a fine thread, which stains more faintly than they do, so as to form a small, loosely folded skein. Part of this skein rests on the achromatic fibres of the spindle. These fibres are doubtless intranuclear in origin. The spindle has centrioles which stain deeply with Heidenhain's hæmatoxylin; and there are the usual terminal radiations in the cytoplasm (fig. 1).

In my next stage, which follows very closely upon the above, the nucleus is almost completely dissolved, all that remains of it being two or three karyosomes still unabsorbed by the cytoplasm. The spindle is now in anaphase (fig. 2). It shows a good number of deflected radiations, in this much resembling one figured by Brasil (1905), but here one can see a well-marked centrosome. I could not demonstrate a centriole. The number of the chromosomes is four. In metaphase (fig. 3) it is impossible to count the chromosomes, and there appear to be more than four, but in the anaphase they can generally be counted, and as I have seen numbers of nuclei in this phase I am in no doubt as to the number.

This appearance of the first spindle agrees in all essentials with the events described by Cuénot (1900), Brasil and

others in the formation of the first spindle in the Gregarines; but most closely with the facts in *Monocystis ascidiæ* (R. Lank.), Siedlecki (1900), and those in *G. ovata* as described by Schnitzler (1905), for here there is no vesicle.

The divisions proceed very rapidly; still, I was fortunate enough to find a cyst with only two nuclei, both, however, dividing (fig. 3).

In a stage with a small number of nuclei it could be seen that in the late anaphase the spindles stretch out, and become, consequently, very much attenuated in the middle. Thus the two daughter-nuclei arising from a division seem, at any rate during the rearrangement of the chromatin, to be surrounded and supported by the wide ends of the spindle of the mother-nucleus (figs. 4 *a* and *b*). Siedlecki (1900) and Brasil record the same method of division in *Monocystids*, and Brasil says that it seems as though the fibres of the spindle helped largely in forming the membranes of the daughter-nuclei. I did not notice that division went on more rapidly at the periphery of the cyst than elsewhere.

In these early stages the centrosomes could be easily demonstrated, and sometimes a sphere could be seen lying completely within the nuclear membrane showing its intranuclear origin (fig. 5). It would seem, too, that the spindle in these divisions is formed within the nuclear membrane, which only disappears when the spindle is fully formed (figs. 5 *a* and 5 *b*). Most of the nuclei contain two or three, some four, spherules of chromatin. These are probably karyosomes, though on account of their small size it is impossible to demonstrate two layers in even the largest of them.

In a few of the cysts, all of about the same age, i.e. having approximately the same number of nuclei, a small sphere of chromatin could be seen being thrust out of each nuclear spindle during division. It is worthy of note that if one spindle in a cyst showed this every other spindle in that cyst did so too. This cannot be regarded as a case of Reduction, for the number of the chromosomes remained

unchanged. I look upon it merely as the casting out of superfluous chromatin, most probably one of the spherules mentioned above, which is quickly dissolved and then absorbed by the surrounding protoplasm (fig. 5, *sph.*).

In some of the cysts intermediate in age between that shown in fig. 5 and the pearl stage there are to be seen some large nuclei at least twice the size of the others. I have never seen these nuclei dividing, but some of them are in a state of degeneration. In the earlier stages all the nuclei divide in exactly the same way. There are not two methods of division as in *Stylorhynchus* (Léger, 1903). These nuclei in the earlier stages seem to be not in any way different from their neighbours; but after having divided a certain number of times they divide no more, then degenerate and die. In the process of degeneration they naturally swell up a little, but their largeness in size as compared with the others is mainly due to their not having undergone so many divisions.

It seems to me that mitotic divisions are continued right on until the "pearl stage," or, as Mr. Cunningham calls it, the "convolution stage" is reached.

Mr. Cunningham, when he wrote his account of *Kalpidorhynchus*, was inclined to think that he was dealing with a case of isogamy. Nevertheless, he found a slight difference between the contents of the gametocytes enclosed in one cyst. I, too, am convinced that there must be an inherent difference between the gametocytes, but in the cysts which I cut this difference was not appreciable till just before the pearl stage was reached. Then, while the dividing-wall between the two gametocytes was still intact, it could be seen that the protoplasm on one side of the wall stained more deeply than that on the other, and that its meshwork was slightly, but only very slightly, finer. It could also be seen that the nuclei on the darker side were rounder, smaller, and darker than the others, showing a more concentrated chromatin. In sections through cysts at the pearl stage a great accentuation of these differences can be noticed. On

the dark or female side the chains of pearls lie on the edges of convoluted bands, which have about half the depth of those on the male side, showing that in the male gametocyte there is much more protoplasm left over after the formation of the gametes than in the female. The nuclei also can be seen in many cases to be about twice as large on the pale as on the dark side (fig. 6). This convinced me that we have anisogamy here, the chief thing which led me to this conclusion being the difference in size between the nuclei of the gametes in the respective gametocytes. But it would not have been easy to prove this difference by means of sections only, for the cysts were cut in all manner of planes. I therefore broke numbers of cysts at random on different slides, and was fortunate enough to isolate in this way a few female gametes from the "pearl stage" and a number of conjugation stages from cysts containing conjugating gametes. Unfortunately I did not so isolate a male gamete, but in the conjugation stages one could see its shape perfectly well.

The female gamete is nearly spherical in shape with a spherical nucleus, which, as a rule, has darker and more concentrated chromatin than that of the male gamete. This nucleus has a volume equal to about one fourth of that of the whole gamete.

The male gamete, on the other hand, consists almost entirely of an oval, sometimes of a pyriform nucleus, which is surrounded by a very thin layer of protoplasm. This nucleus is generally about twice as large as that of the female gamete. The oval nuclei are the more common (fig. 7).

While the nucleus of the female gamete is surmounted by a wide, low cone, the cone on the male nucleus is high and narrow. In both cases the centrioles can often be seen to be double.

In the act of conjugation it seems as though the male nucleus with its cone forces itself through its own protoplasm, which it casts off like a sheath as it enters the female gamete (fig. 7, *b*, *c*, *d*, etc.). This is what happens most frequently,

but I found some conjugations in which there was apparently a fusion of the cytoplasm of the gametes, as well as of their nuclei.

In neither gamete nor zygote could I demonstrate a cell-wall by the use of Delafield's hæmatoxylin, but preparations stained with Licht-Grün and picric acid showed a delicate outline to the cells. This outline was more easily shown in sections than in whole cell preparations.

The zygote is at first pyriform with very little in the way of a stalk, but with one end a good deal thicker and rounder than the other. The cell-wall is slightly more pronounced than it was in the conjugation stage. It is at the narrow, pointed end of the zygote that its nucleus lies. This nucleus is also pyriform and has its wide end directed towards the wide end of the zygote. Its chromatin is loosely arranged in large thick rods and lumps, and is not surrounded by a membrane. The absence of a nuclear membrane here is probably not merely a result of the fusion of the nuclei, but also a means of aiding the expulsion of a vacuole from the nucleus (fig 8, *b*).

Brasil (1905) also notes the expulsion of a vacuole (sphère hyaline) from the nucleus of every zygote in a cyst; and, as well as the vacuole, he saw extruded a small globule of chromatin, which he conjectures may form part of that chromatin which is subsequently to be seen at both ends of the spores of *Monocystis* after the first nuclear division. I saw no such extrusion of a grain of chromatin here, but on the assumption that it is merely superfluous chromatin this is not to be wondered at, for a small globule of superfluous chromatin was ejected at an earlier stage (see above and fig. 8).

The absence of a nuclear membrane may possibly facilitate the movements of the nucleus, for it certainly does move. One can see the vacuole forming, and after its extrusion the nucleus has not only acquired a membrane, but now lies at the wide end of the zygote. Nuclei can be seen in intermediate positions during the formation of the vacuole. Its

extrusion and the formation of the membrane seem to take place simultaneously.

After this the nucleus becomes approximately spherical, and its chromatin appears to be more finely divided and more closely packed than it was. The zygote at this stage often has a stalk-like projection at its narrow end, and this stalk persists so that the spore has the shape of a pear with a thickened stalk. Sometimes this stalk-like projection or elongation does not appear till later, but it is invariably present in the stage with four nuclei. The nucleus now divides into two, then into four, and ultimately into the eight nuclei of the sporozoites. It is my belief that at any rate the earlier of these divisions are mitotic, but I have not been able to prove this satisfactorily, and Mr. Cunningham is not of my opinion. It is at the stage with one spherical nucleus at the wide end of the cell that the cell-wall becomes thickened to form the sporocyst, and the zygote thus becomes a spore. Mr. Cunningham has mentioned the transparency of this sporocyst. I was able to see it, in preparations stained and mounted in spirit, as a dark line which follows the outline of the cytoplasm very closely. Rarely, until the spores are fully ripe, i. e. until the cytoplasm is segregated round each of the sporozoite nuclei, does it leave the little stalk-like projection of the sporocyst (fig. 8).

In some of my preparations the ripe spores are burst. This may be due to reagents, the withdrawal of the cytoplasm from the stalk having left a spot vulnerable to pressure in the sporocyst. But it may be the natural course of events, for the sporocyst seems to fit the cytoplasm fairly tightly, and the withdrawal of some of the cytoplasm from the stalk into the body of the spore may have caused the sporocyst to split.

The sporozoites are vermiform, with pointed ends. The long nucleus occupies at least half the volume of each individual. The chromatin is finely divided and evenly distributed throughout the whole nucleus (fig. 8).

It seems to me that it is only by following this chromatin

in the nuclei of the stages between sporozoite and trophozoite, and in watching the evolution of the karyosomes, that we can arrive at any conclusion as to the origin of the chromatin of the chromosomes in the first spindle. With this end in view I examined the alimentary tract of several infected specimens of *Arenicola ecaudata*, and found cysts in the œsophagus and in the intestine, which shows that swallowing is a possible method of infection. I have also cut sections through the gut walls of several specimens in the hopes of finding sporozoites in transit, but always without success. It seems most likely that the sporozoites make their way very speedily through the gut walls and then carry on their further development in the coelomic fluid. I did find in the coelom specimens of a very young trophozoite without an epimerite (fig. 9); but this, unfortunately for my purposes, had already several large and small karyosomes in its nucleus.

Two other points on which I have been able to supplement Mr. Cunningham's observations are multiple association and the structure and reproduction of the karyosomes.

Multiple Associations.—In preparing the cysts for embedding I noticed many cases of multiple association—sometimes there were as many as five individuals together, sometimes four, and very frequently three. Mr. Cunningham has figured four trophozoites coming together (1907). On cutting the sections I found a cyst containing five gametocytes, each of which had many nuclei; and I found several cysts with three gametocytes in like condition. But since I have never found a cyst containing more than two gametocytes in the "pearl stage" or further advanced, I am forced on to Dr. Woodcock's conclusion (1906) that these multiple associations come to nothing.

STRUCTURE OF THE KARYOSOME.

Unfortunately I have not succeeded in tracing the origin of the first karyosome. In the youngest trophozoite seen by me (fig. 9) there are already several karyosomes in the nucleus.

The larger karyosomes all consist of two layers—an outer dense layer which stains deeply and strongly with Heidenhain's hæmatoxylin and other chromatin stains, and is, in fact, basophile, and an inner part which has not so strong an attraction for basic stains, stains palely with Heidenhain, and strongly with acid stains such as orange G., Licht-Grün and picric acid, etc. The outer layer is, of course, chromatin, and the inner is nucleolar substance or plastin. The smaller karyosomes contain no plastin. They consist wholly of basophile chromatin. This, it seems to me, is only an expression of the fact that, as the karyosome increases in age and size, it becomes by degrees converted from a basophile into an acidophile substance—i. e. from chromatin into plastin. The larger karyosomes are often divided up into a number of small chambers, each chamber being surrounded by a wall or walls of chromatin. In fig. 10 there is a karyosome in which this process is beginning. This drawing shows the nucleus of a gametocyte, but the nuclei of trophozoites often contain karyosomes in the same condition. There can be little doubt that the chromatin of the partitions and of the little knob-like thickenings is derived from the dense outer layer. This turning in, so to speak, of the chromatin may take place in order to increase its area of action, for the result is an increase in the quantity of the plastin, and ultimately this increase is at the expense of the chromatin. I have seen karyosomes in which there were larger chambers with thinner walls, and others from which the outer rim of chromatin had gone completely. It seems as though finally the whole karyosome becomes converted into plastin.

The karyosome divided up into chambers resembles on a small scale the karyosome of *Aggregata* as described by Moroff (1908).

REPRODUCTION OF THE KARYOSOMES.

Increase in the number of karyosomes takes place by a kind of internal budding from the chromatin layer. I have

not seen a single case of scissiparity. The buds (fig. 10) consist entirely of chromatin, and it is not until some time after their escape from the parent karyosome that the inner layer (plastin) makes its appearance in them.

On first noticing these internal buds I was puzzled as to how they made their escape, but soon came to the conclusion that an exit was made as occasion demanded and then closed up again. I was therefore much pleased to find that Schneider had described the same kind of internal budding in his account of *Klossia (Aggregata) eberthi* as long ago as 1883. See also Schellack (1907).

At present, affinity for different stains is our usual criterion for differentiating the contents of the cell, and we make a broad distinction between chromatin and cytoplasm by saying that one is basophile and the other acidophile.

In working at this gregarine my first staining operation was generally the use of Heidenhain's hæmatoxylin, and I could not help noticing that in staining strongly with an acid stain, after using Heidenhain, the inner part of the karyosome, the linin meshwork and the centrosomes all took up the acid stain (eosin, orange G., or picric and Licht-Grün), appearing to be stained by that and by nothing else. The centrioles and outer layer of the karyosomes and the chromosomes, however, kept black and were not affected by the acid stain at all. If I stained weakly with the acid stain the meshwork, inner part of the karyosome, and the centrosomes all retained the black stain of the Heidenhain, though the black on the inner part of the karyosome might with more accuracy be called grey. I could, in fact, vary the amount of greenness or blackness by varying the intensity of my acid stain, but one or other always predominated. Now the linin meshwork is known to consist partly of protoplasm and partly of chromatin. In the inner part of the karyosome chromatin is being converted into plastin, presumably for the nourishment of the nucleus and ultimately of the cytoplasm. Does it not seem that while this process of conversion is going on, there must be in the inner part of the karyosome a mixture of

acidophile plastin and basophile chromatin, and that in the centrosomes also there is a mixture of chromatin and an acidophile substance? On this supposition we can, at least, give our explanation of the results of staining; for it would seem that when (after using Heidenhain) we stain strongly with an acid stain, then in the resulting preparation the protoplasm masks the chromatin; on the other hand, when (after Heidenhain) we stain weakly with an acid stain or do not use one at all, the chromatin masks the protoplasm. The chromosomes, centrioles and outer part of the karyosomes, since they consist entirely of chromatin, when once stained with Heidenhain keep their black appearance unaltered by any subsequent treatment with acid stains.

In conclusion, I wish to express my thanks to Professor Minchin for his friendly advice as to literature, and also for his criticism of this paper.

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EXPLANATION OF PLATE 29.

Illustrating Miss M. Robinson’s paper “On the Reproduction of *Kalpidorhynchus arenicolæ* (Cnghm.).”

Fig. 1.—The first nuclear spindle and the breaking up nucleus of the gametocyte. $\times 1200$.

Fig. 2.—The first nuclear spindle. Anaphase. $\times 1050$.

Fig. 3.—The second and third nuclei. Metaphase. $\times 1050$.

Fig. 4.—(a) Spindle drawn out during late anaphase. (b) Two daughter-nuclei resulting from above. $\times 1200$.

Fig. 5.—Part of a cyst in section, showing nuclei in different states of division. $\times 1200$.

Fig. 5a and 5b.—Two nuclei from same cyst as fig. 5, showing formation of the spindle within the nuclear membrane. $\times 1200$.

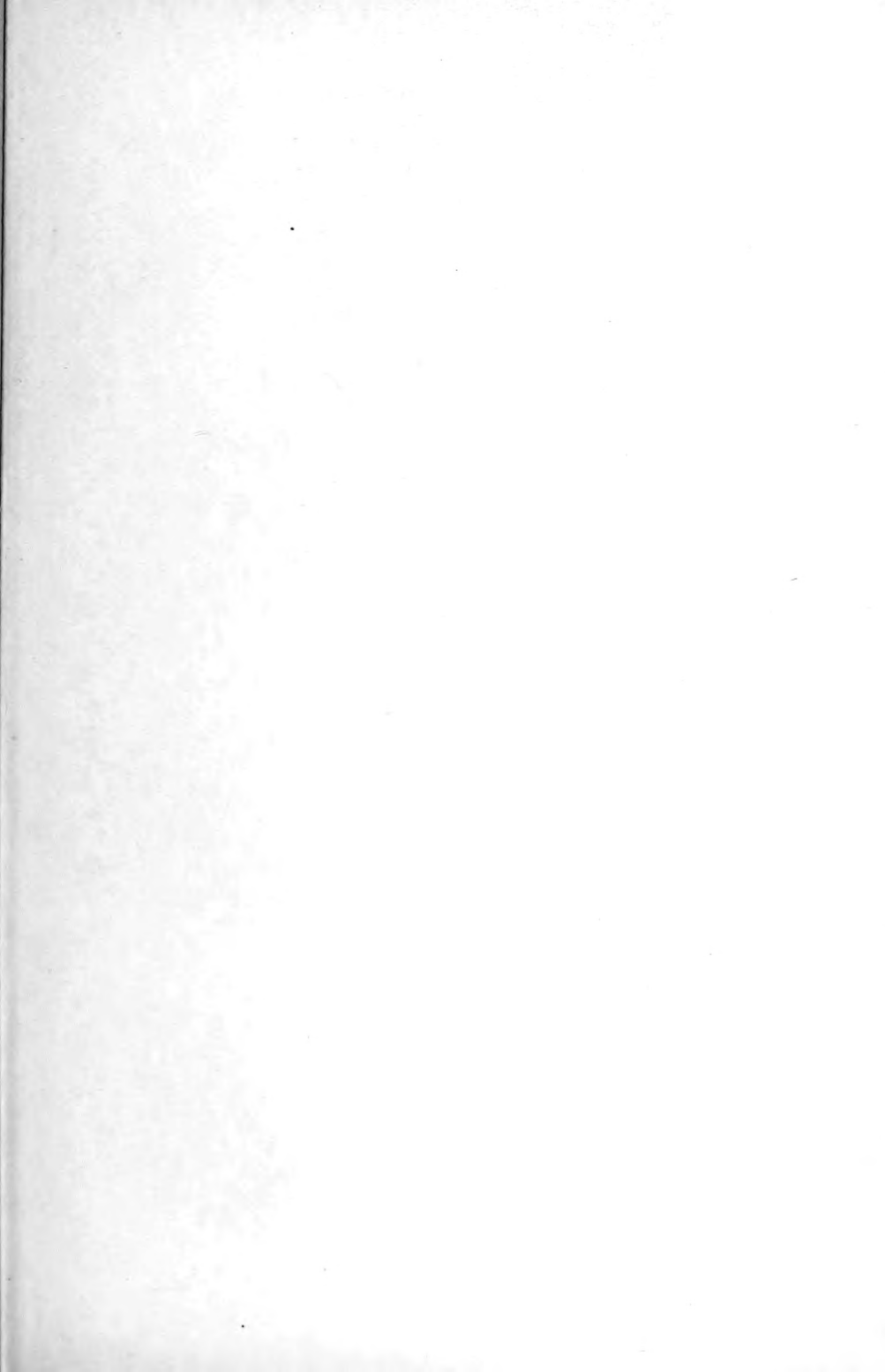
Fig. 6.—Portion of cyst in section at the pearl stage. $\times 500$.

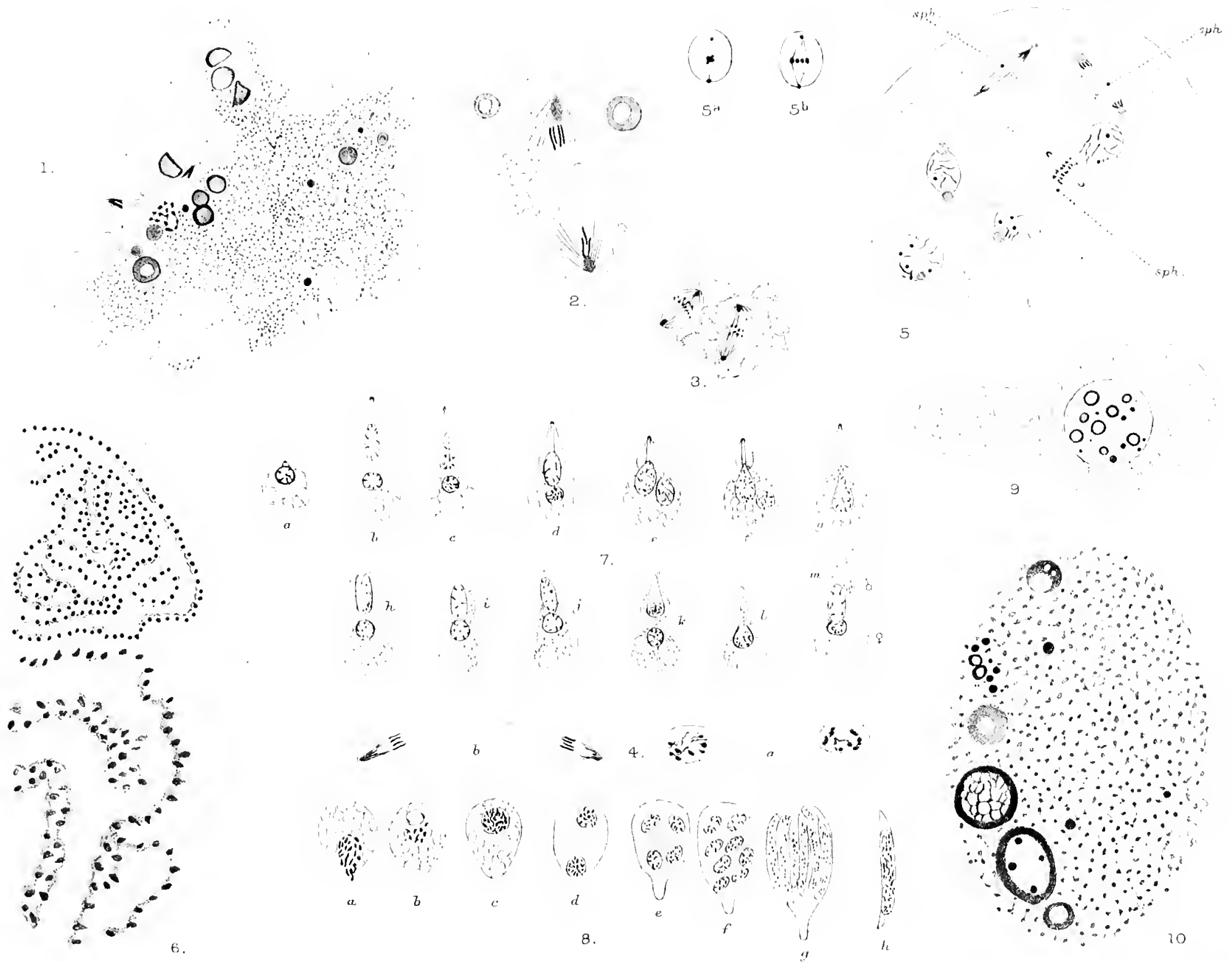
Fig. 7.—(d) Female gamete. $\times 1250$. (b—g) Conjugation stages stained with Heidenhain, Licht-Grün and picric acid. $\times 1250$. (h—m) Conjugation stages stained with Delafield’s hæmatoxylin. $\times 1250$.

Fig. 8.—(a) Zygote soon after conjugation. (b) Zygote with nucleus extruding a vacuole. (c) Spore—one nucleus stage. (d) Spore with two nuclei. (e) Spore with four nuclei. (f) Spore with eight nuclei. (g) Spore with eight nuclei. (h) Sporozoite. $\times 2000$.

Fig. 9.—Young trophozoite without an epimerite. $\times 500$.

Fig. 10.—Nucleus of a gametocyte showing multiplication of the karyosome, and division of a karyosome into chambers. $\times 2000$.







Studies in the Experimental Analysis of Sex.

By

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With Plate 30.

1. ON MENDELIAN THEORIES OF SEX.

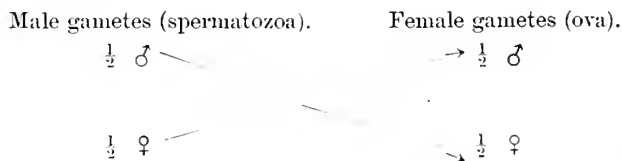
THE re-discovery of Mendel's observations on heredity, and the extended application of his ideas by such writers as Correns, Tschermak, and Bateson to every branch of life, has had a very profound influence on contemporary biological conceptions, and it is not surprising that the problem of sex, which has occasioned so many speculative theories in past times, has been brought under the focus of Mendelian research and subjected to its analysis. The conceptions of segregation, of allelomorphism, of heterozygotism, to employ the accepted terminology of Professor Bateson (1), seem admirably suited in their application to the phenomena of sex, because in sexual reproduction we actually see that the sexual characters do segregate into two sharply separated sets of individuals, the males and the females, as if maleness and femaleness were in some way allelomorphic to one another, while the occurrence of hermaphrodite forms and the latent presence in one sex of characters proper to the opposite sex indicate the phenomenon of heterozygotism or sex-hybridism.

There is a further reason why Mendelian speculation has naturally turned in the direction of sex. So long as it was held that the sex of any animal or plant was not a question of inheritance or of a pre-determined quality of the germ,

investigators principally occupied themselves with statistics, and with the supposed influence of various external factors, such as food or temperature, upon the production of one sex or the other. But the almost wholly negative result of such investigations, and the positive evidence afforded by such cases as that of the bee, and of the rotifer, *Hydatina*, in the latter of which two structurally different kinds of eggs are produced destined to give rise to males and females respectively, have influenced biologists to look for the cause of sex-determination in the mechanism of heredity.

The first to definitely formulate a Mendelian theory of sex was Castle in 1903 (2). He supposed that in all cases each sex is a sex-hybrid or heterozygote of the composition ♂ ♀, but that in the male sex maleness was dominant, and in the female femaleness. As evidence that in each sex the opposite sex-character is present in a latent state, he adduces the undoubted transmission of male characters through the female and vice versâ, and the appearance of the secondary sexual characters of the opposite sex in animals, as the result of injury or disease of the gonads.

The process of segregation and fertilisation he conceives as follows: Each sex being a heterozygote produces male and female gametes in equal proportions; but among them there is selective fertilisation of such a kind that only male-bearing spermatozoa can fertilise female-bearing eggs and vice versâ. Thus:



Both the zygotes produced have thus the composition ♂ ♀, and we are given to understand that for some reason in half the zygotes maleness dominates, and in the other femaleness.

It must be admitted that this theory, according to which every individual is potentially hermaphrodite, is sufficiently

comprehensive to cover the fact, but it involves two large assumptions for which there is little or no evidence, and its very comprehensiveness prevents its affording any satisfactory explanation of the undoubted variations in sexual constitution which occur in Nature.

The two assumptions, firstly, that selective fertilisation occurs, and secondly, that 'dominance is reversed in two sets of individuals in the same species, for some unknown reason, are not beyond the bounds of possibility, but they are unwarranted and cumbrous additions to Mendelian theory which we would gladly avoid, at any rate until all attempts at a simpler explanation have failed.

The essence of a simpler interpretation was first hit upon by McClung in 1902 (3), although he did not express himself in Mendelian language or definitely formulate a Mendelian theory of sex. McClung observed that in certain kinds of insect the males produced two forms of spermatozoa in equal numbers, one half the spermatozoa containing an accessory chromosome and the other half lacking it. This peculiar distribution of the accessory chromosome was found to be effected in the maturation division of the spermatocytes, the accessory chromosome, instead of dividing, passing bodily over into half the secondary spermatocytes. In the maturation of the ova no accessory chromosome was observed. McClung clearly pointed out that the behaviour of this chromosome in the male was in some way connected with the determination of sex. He considered that the spermatozoa containing the accessory chromosome on fertilising an egg gave rise to a male, while the normal spermatozoa gave rise to females.

It is clear that, although McClung did not state it in this way, we have here the essence of a Mendelian theory of sex, according to which the male is a heterozygote of composition, ♂ ♀, giving rise to ♂ and ♀ gametes, while the female is a recessive ♀ ♀, giving rise to pure ♀ gametes. This would further account for the production of the two sexes in equal proportions according to the laws of chance. Unfortunately this simple account of the matter has been proved

erroneous, especially by E. B. Wilson and his pupils. Wilson (4) has shown that although the dimorphism of the spermatozoa does occur, and is, indeed, a frequent phenomenon among insects, yet that where an "accessory" chromosome is present in the males it is due to half the spermatozoa containing one chromosome less than the eggs, while those with the "accessory" chromosome contain the same number as the eggs. It is therefore impossible to say that the spermatozoa with the accessory chromosome give rise to males, as it is evidently those without the accessory chromosome which, in conjunction with an egg, produce an animal with an odd number of chromosomes, i.e. a male. This fact has prevented Wilson hitherto from accepting McClung's simple interpretation, since he is convinced that the sex-character must be represented by a definite chromosome in the cell. Of course, if we are content to give up the idea of the sex-character being necessarily represented by a definite chromosome, an idea which, indeed, has not very much to support it, McClung's explanation would still hold, except that the male-producing spermatozoa would be those without the accessory chromosome, while the female-producing spermatozoa would be those that possessed it. Professor Wilson, however, will not readily accept this, and he has endeavoured to get over the difficulty by framing several different theories of sex of very great complication. The latest of these theories not only assumes a great complication of gametic representatives, but also involves selective fertilisation, so that it belongs to the same category as Castle's theory and need not be discussed further at present.

During the years 1904-6 I was occupied at Naples in studying the effect of the parasitic Cirripede *Sacculina* on its host, a species of spider crab (*Inachus*), and from this study I was led shortly to formulate a Mendelian theory of sex. As the result of the examination of many thousand specimens of the crab at various stages of infection, I concluded that whereas the males, under the influence of the parasite, were capable of assuming all the female secondary

sexual characters, and under certain conditions might even develop typical ova in their testes, the infected females, on the other hand, although the ovaries in many cases completely disappeared, never approached to the male primary or secondary sexual characters in the slightest degree. After citing and examining other cases, especially among the Crustacea, in which the male sex showed undoubted signs of latent hermaphroditism, I concluded that, at any rate in these forms, it would appear that the male was a potential hermaphrodite, and the female purely female. The significant bearing of this conclusion upon a Mendelian theory of sex was obvious, and since at the time the only definitely formulated Mendelian theory of sex was that of Castle, according to which both sexes of any species were potentially hermaphrodite, I was led to formulate an alternative theory, which did not require the assumption of selective fertilisation, in the following words: "There is a final topic for discussion, namely, the connection between the theory of sex here adopted and contemporary Mendelian theory. It is interesting to observe that where an attempt has been successfully made to find structural differences in the germ-cells as possible indications of this early sexual differentiation, the manner of this differentiation is in harmony with the results we have obtained from the study of parasitic castration. The discoveries of Henking, McClung, Wilson and others have shown that in many insects two kinds of spermatozoa exist, differing in the constitution of their chromosomes, while the eggs are apparently all the same. If we suppose that the two kinds of spermatozoa represent the male and female sex respectively, while the eggs are purely female, we would obtain in the process of sexual generation $\frac{1}{2} \text{ ♂ } \text{ ♀ } + \frac{1}{2} \text{ ♀ } \text{ ♀ }$, in which the male spermatozoa united with female eggs give rise to males of really hermaphrodite constitution, while the female spermatozoa united with female eggs give rise to females of pure female constitution. It is obvious that this interpretation is in strict agreement with the main conclusion brought out in this chapter, viz. that males are potentially hermaphrodites,

while females are incapable of assuming male characters. It is doubtful, however, whether this particular 'Mendelian' interpretation can be applied generally, because in some animals, e. g. the bee, it appears that the egg by itself is male and only becomes female through fertilisation, while in many Cladocera and Aphides females give rise parthenogenetically to males. It appears, therefore, that the primary mechanism of sex determination may be variously distributed in the germ-cells" ([5] "Rhizocephala," 'Fauna and Flora des Golfes von Neapel,' Monogr. 29, p. 89, 1906).

In the above words I believe that the following Mendelian theory of sex is clearly stated :

(1) That in certain species of animals (e. g. *Inachus* and other Crustacea) the male is a heterozygote of the composition ♂ ♀, while the female is a pure recessive of the composition ♀ ♀.

(2) That the sexual constitution is not necessarily the same in all species of animals, e. g. in the case of the Cladocera, where the female is proved to be a heterozygote owing to her capacity for producing both males and females parthenogenetically.

In excuse for recapitulating these statements it may be pleaded that, owing to their appearance in a publication principally devoted to morphological studies, they have not been referred to by subsequent writers who have independently arrived at the same Mendelian theory of sex.

Thus, Professor Bateson and Mr. Punnett (1908), in offering an interpretation of the striking results obtained by Doncaster in his breeding experiments with the currant moth, have suggested that in this case the female is a heterozygote (♂ ♀) and the male a homozygote (♂ ♂). The reason for this interpretation is as follows: The common currant moth, *Abraxas grossulariata*, occasionally gives rise to a pale-coloured variety, *lacticolor*, and this variety has been hitherto supposed to be confined to the female sex. Doncaster made, among others, the following crosses :

(1) *Lacticolor* ♀ × *grossulariata* ♂ gave ♂ and ♀

all *Grossulariata*. This is the F_1 generation used in succeeding crosses.

(2) F_1 *grossulariata* ♀ × F_1 *grossulariata* ♂ gave F_2 *grossulariata* ♂s and ♀s and *lacticolor* ♀s, but no *lacticolor* ♂s.

(3) *Lacticolor* ♀ × F_1 *grossulariata* ♂ gave *grossulariata* ♂s and ♀s and *lacticolor* ♂s and ♀s, the *lacticolor* ♂s being the first ever seen.

The explanation of these results offered by Bateson and Punnett depends on the two assumptions—

(1) That the female is heterozygous in sex, femaleness being dominant, and the male a homozygous recessive.

(2) That when in F_1 the two dominant characters, femaleness and the *grossulariata* factor co-exist, there is spurious allelomorphism or repulsion between them, such that each gamete takes one or other of these factors, not both.

The three crosses described above read, therefore :

(1) Lac. ♀ × Gros. ♂

Produce gametes—

Lac. ♀. Lac. ♂ × Gros. ♂

Produce zygotes—

F_1 Lac. ♀ Gros. ♂. Lac. ♂ Gros. ♂.

(2) F_1 Gros. ♀ × F_1 Gros. ♂

Produce gametes—

Lac. ♀. Gros. ♂ (Lac. ♂) (Gros. ♀) × Lac. ♂ Gros. ♂

Produce zygotes—

Lac. ♀ Lac. ♂. Lac. ♀ Gros. ♂. Gros. ♂ Lac. ♂. Gros. ♂

Gros. ♂.

The gametes in brackets are not formed owing to spurious allelomorphism.

(3) Lac. ♀ × F_1 Gros. ♂

Produce gametes—

Lac. ♀ Lac. ♂ × Gros. ♂ Lac. ♂

Produce zygotes—

Lac. ♀ Gros. ♂. Lac. ♀ Lac. ♂. Lac. ♂ Gros. ♂.

Lac. ♂ Lac. ♂.

This remarkable case has been given in full, as it illustrates

the kind of way in which light is thrown on the constitution of sex by breeding experiments. This is by no means the only interpretation of the facts that could be offered, but it is the simplest and the most in accordance with other results in which the phenomenon of spurious allelomorphism appears to occur. It must be remembered, however, that in cases of this kind we are not dealing with the sex characters directly, but only through the medium of an assumption which certainly gives a simple though not the sole possible explanation of the results.

A similar interpretation is given by Bateson (1) in the case of the cinnamon canary and the brown Leghorn fowl.

Professor Correns (8), on the other hand, as the result of hybridisation experiments with *Bryonia*, comes to the conclusion that in this case the male is heterozygous and the female homozygous, and he inclines to give this interpretation a wide application both in the animal and plant kingdom.

It would appear, therefore, that a considerable body of evidence is accumulating, drawn from very various fields of research, which tends to show the justness of the view that in sex we are dealing with a phenomenon which may be termed "half-hybridism," i. e. one sex, either male or female, is always a sex-hybrid, while the other is pure.

We may now examine some of the serious difficulties which this view encounters. In the first place it may appear very strange that the sex-hybrid individual (σ φ) should appear in one case as a male and in another as a female, in other words that there should be such a complete reversal of dominance. But it may be pointed out that dominance is one of the least constant phenomena in cases which have yielded satisfactorily to Mendelian analysis. Let us, moreover, consider what happens in the case of functionally hermaphrodite animals, in which there can be no doubt at all as to their heterozygous nature. We may divide such animals into protandrous, simultaneous, and protogynous hermaphrodites, of which the first category is by far the commonest. To take a typical instance of protandry, in the parasitic Isopoda Epi-

carida, the hermaphrodite individuals are at first, in the larval state, apparently pure males. They then settle down as parasites and lose every trace of their male organisation and become converted into what are apparently pure females. In fact for a very long period of time they were considered by naturalists to be typically dicecious animals with very marked sexual dimorphism. Now let us suppose that for some reason or other certain of these individuals failed to develop further than the male larval state. They would then be constitutionally hermaphrodites, in which the male condition was dominant, and they would be put down as males. Then let us suppose that other individuals for some reason left out the male period of their history, possibly by becoming fixed parasites at an earlier period before the testes were developed. These individuals would then be constitutionally hermaphrodites, in which the female condition was dominant, and they would be considered with equal confidence as females. In this way, by shifting the period at which the sexual organisation matures, a process which may be very easily conceived to occur, we would arrive at an apparently complete reversal of dominance.

That this shifting of the period of maturity actually occurs is shown by the existence of the three classes of hermaphroditism noted above; thus in the single class of simple Ascidians we meet with both protandry and protogyny. Let us take another slightly different instance, the case of the spider crab *Inachus*, parasitised by *Sacculina*. The male parasitically castrated crabs may show every degree of modification towards the female state, until finally we obtain male crabs which have been so completely transformed as to retain only a single male character, viz. the copulatory style in so reduced a state as to be invisible except with a lens. These crabs, besides exhibiting in a typical condition the broad abdomen, reduced chelæ, and abdominal swimmerets of the female, may under certain conditions develop ova from the remains of their testes, and these ova may grow to a very large size and become filled with the reddish-coloured food-yolk character-

istic of the species. In these cases, although a certain amount of sperm was always present as well, the female part of the hermaphrodite gland greatly preponderated. Here, then, we have individuals of hermaphrodite constitution, which normally only show the male characters throughout life, i. e. in which maleness is dominant; but when the presence of the parasitic *Sacculina* sets up a disturbance this dominance is almost completely reversed, and the hitherto recessive female characters appear in all completeness.

Again, to take the opposite case. In deer and pheasants it is well known that certain individuals which have actually bred as females, may in old age develop the male secondary sexual characters in a very complete manner. Such individuals prove themselves to have been heterozygotes in which the dominant female character is replaced for some reason by the recessive male.

It is clear, therefore, from the foregoing instances, that individuals of hermaphrodite constitution may exhibit any of a whole series of modifications from apparently pure maleness, through simultaneous hermaphroditism, to apparently pure femaleness. This being the case, the difficulty of considering that in a normally diœcious animal either one sex or the other is a sex-hybrid, according to the species or group of species we are dealing with, is materially lessened. We may, in fact, state the case as follows: that three types of individuals exist in respect of sex, pure males, hermaphrodites, and pure females, and that the hermaphrodites may appear as males, hermaphrodites, or females according to a physiological condition which is confessedly not understood.

We have, so far, formulated the Mendelian theory of sex, so as to account for the existence within a species of individuals having either the constitution ♂ ♀ and ♀ ♀ or of ♂ ♀ and ♂ ♂, in the former case maleness being dominant, in the latter femaleness. This is the simple half-hybrid theory of sex. We must, however, consider the possibility of the existence of three types of individuals within the same species, viz. ♂ ♂, ♂ ♀, and ♀ ♀. The difficulty of this

conception lies in the fact that we would have to assume that some of the heterozygous individuals (σ ♀) would have to function as males and others as females in order to secure a continuous output of pure males and females. For if all the heterozygous individuals were functionally either males or females exclusively, they could only produce one form of pure zygote in combination with the gametes of the pure sex. The supposition that the heterozygote may appear as either male or female in the same species of animal is by no means impossible, since it only leaves us face to face with the same problem, which is at the root of the whole sex question, namely, What is the physiological state which brings about the suppression or development of the male or female characters in animals which undoubtedly possess the potentiality of both? It is held by a number of observers, for instance Mr. Walter Heape (9), that the proportional output of the sexes is influenced to a very large extent by external conditions of feeding, temperature, breeding-seasons, etc., and it is quite possible that these influences are sufficient to give a bias to the heterozygous embryo to appear as either male or female.

However this may be, the assumption that three types of individuals may exist with distinct gametic output would account for the marked disproportion in number of the sexes, which is known to occur in the offspring of certain families, both among animals (especially butterflies) and human beings.

There is another class of facts which offers an interesting but difficult field for interpretation upon Mendelian lines, viz. the sex of parthenogenetically produced offspring. In the case of such animals we get every variety of product, the parthenogenetically produced young being either only males or only females or a mixed brood of both sexes. But there are certain common features in these cases. Thus, in the case of the bee, in Cladocera, and in certain Rotifera the fertilised eggs always give rise to females, the males being only produced parthenogenetically. The females in some of these cases, e. g. the Cladocera, are proved beyond doubt to be

heterozygotes, as one and the same female may give rise parthenogenetically to males and females. It is also proved in these cases that no segregation occurs in the production of parthenogenetic females, since a parthenogenetically produced female may give rise by parthenogenesis to a mixed brood of males and females. We may be certain, therefore, that the females in these cases are heterozygotes (δ η). With regard to the parthenogenetically produced males we are naturally more in the dark, since they produce no parthenogenetic young by which they can be judged. The most obvious supposition is that, since they, like the females, are produced parthenogenetically, there is no segregation in their production, and that they also are heterozygous (δ η) and produce δ and η spermatozoa. If, however, we are going to maintain the half-hybridism theory of sex, since the females are certainly δ η the males must be pure δ δ , and some process of segregation must occur. But if this is the case why do the eggs, when fertilised with such purely male spermatozoa, invariably give rise to females? It has been held, especially for the bee, that the mere act of fertilisation in itself is the cause of the production of females, and if this is the case it is very difficult to bring the phenomenon into any relation with Mendelian theory. There is, however, an alternative to this explanation. We may legitimately hold that the female gives rise to two different kinds of eggs, male and female, of which only the female eggs are capable of being fertilised. Such female eggs, being fertilised by the male spermatozoa, will give rise to heterozygotes of the composition δ η , which ex hypothesi will appear as females, while the unfertilised male eggs will give rise to males of pure male constitution.

In the Rotifer *Hydatina* and in the worm *Dinophilus* we know that two different kinds of eggs are produced, large eggs which give rise to females and small eggs which give males, and this fact seems at first to favour the theory proposed above. In the case of *Dinophilus*, however, Dr. Shearer, in a recent unpublished research, has shown that the female *Dinophilus* is fertilised while still immature

and before any visible differentiation of the eggs into male-producing and female-producing forms has occurred, so that this differentiation may be the result of fertilisation and not due to the inherent heterozygotism of the female. In the case of *Hydatina*,* as clearly shown in R. C. Punnett's interesting paper (10), and in the Cladocera, there is no doubt that the female can give rise parthenogenetically to both males and females, so that in these cases the female sex is certainly heterozygous; but we have no certain means of judging whether it is the female eggs alone which are capable of being fertilised.

Interesting and suggestive as the evidence drawn from breeding experiments and from the cytology of maturation is, it appears that the most cogent and unassailable evidence for sex heterozygotism is afforded firstly by *Inachus* parasitised by *Sacculina*, in which the male sex is proved to possess the secondary and primary female characters in a latent state, and secondly by the Cladocera, in which the female sex is proved to be heterozygous owing to the parthenogenetic females giving rise to both males and females.

The foregoing arguments and considerations have shown us that while a number of facts definitely support the half-hybrid Mendelian theory of sex, there is nothing which definitely controverts it. The theory has the merit of being a simple one, and it accounts for the facts without the necessity of making any additional assumptions such as that of selective fertilisation, an assumption which may in the future prove necessary, but which would seriously impair the validity of Mendelian analysis.

And it may be remarked that the half-hybrid theory of sex not only alters our view of the sexual constitution of animals and plants, but it indicates, if it is well founded, the real ground upon which the problem of sex must be attacked. This, as has been already stated, is the inquiry as to the physiological conditions under which one sex or the other gains the upper hand, i. e. becomes dominant in a heterozygous individual which contains potentially the elements of both sexes.

It must be remembered, moreover, that sex is not necessarily a simple unit character, inherited in its entirety as such; thus sexual characters fall into two main divisions, primary and secondary, and the latter again may variously affect any of the organs or parts of the body. We will give reasons, however, in the next section, for assuming the existence of a sexual formative substance, male or female, which controls the development of both primary and secondary sexual characters, and for the present it is assumed that the male and female modifications of this substance are the allelomorphs which segregate in the manner described above, and give rise to the half-hybrid nature of sex.

2. ON THE CORRELATION BETWEEN PRIMARY AND SECONDARY SEXUAL CHARACTERS.

Various definitions have been given of primary and secondary sexual characters. In these studies the term "primary sexual characters" is applied to those characters which affect the differentiation of the actual generative organ, testis or ovary, in which the ova and spermatozoa are produced, while all those sexual characters are considered secondary which affect the other parts of the body, e.g. generative ducts, copulatory or any other organs, external or internal, which differ in the two sexes.

The fact that there is a physiological correlation between the state of development of the secondary sexual characters and the primary reproductive gland has been vaguely recognised from time immemorial. Thus the knowledge that the castrated males of the human species and of many races of domestic animals show in various degrees an arrested development of the secondary sexual characters goes back to periods long antecedent to scientific biology. But despite this long familiarity with certain fundamental facts, there does not exist even at the present time a clear conception of the nature and limits of this correlation. Careful observation and a certain amount of experimental work have revealed a

very large body of facts bearing upon the question, but they have chiefly served to emphasise the irregularity of the phenomenon, and it is certainly impossible at present to formulate any definite theory to connect the known facts in a comprehensive and satisfactory manner. It is not the purpose of this essay to attempt a review of the recorded cases of so-called hermaphroditism and of the abnormal condition of the sexual system which throw a somewhat fitful light on the problem, but reference may be made to the critical work of Herbst ('Formative Reize' [11]), in which he discusses a large body of conflicting evidence and draws certain wide conclusions. He shows that, while the evidence in favour of some causal correlation existing between the primary and secondary sexual characters is overwhelming, yet this correlation is not of so definite a nature as to sanction the simple view that the development of the secondary sexual characters as a whole is directly dependent on the development of the primary characters; he concludes, however, definitely, that in the vast majority of cases the full development of the secondary sexual characters in either sex is conditioned by the presence of the corresponding primary organ in a functional state. Even this very cautious and limited acceptance of correlation breaks down in certain exceptional cases. Thus Kellogg (12) has shown that the gonad of the silkworm can be extirpated in the larval stage, so that no trace of this organ is to be found in the adult, and yet the moth develops its marked secondary sexual characters to the full, while Meisenheimer (13) has performed the ingenious experiment of transplanting the young gonad from one sex into the other where it may develop to maturity, and yet no change is to be observed in the secondary sexual characters of the adult insect. In the case of those particular insects, therefore, it appears that there is no connection whatever between the development of the secondary sexual characters and the presence of a differentiated gonad, and though it is true that this is the only case known in which this entire independence is to be observed, yet we can trace a series of

instances in which the removal of the gonad inhibits in greatly varying degrees the development of the secondary characters.

In attempting, therefore, to frame a theory which shall give a satisfactory account of the undoubted correlation which exists in various degrees between the primary and secondary sexual characters, we must bear in mind the variability of this correlation, and even in certain cases its non-existence. Mr. J. T. Cunningham, in a recent interesting paper (14), has put forward a theory which appears to me to fail in this respect. According to his theory the development of the secondary sexual characters is due to the action of an internal secretion produced by the gonad, principally at its maturity. There can be no doubt that this statement is partially true, but it does not cover all the facts. If it represented the whole truth the absence of a differentiated gonad should in all cases be accompanied by the entire absence of all secondary sexual characters usually connected with it, and this is certainly not the case.

We will now examine in some detail a particular instance which appears to throw a more definite light on the subject than any that has hitherto been obtained. The discovery of the phenomenon of parasitic castration was made by the late Professor Giard, and it always seemed to me very surprising that no one had followed up his discovery, since it affords a very obvious and simple way of gaining an insight into the nature of sex, without the necessity of performing a delicate operation with the clumsy means at our disposal. For in this case, instead of performing the operation ourselves, we find that Nature employs for the purpose some of the lower classes of creation, who, though not endowed with the great intelligence which is sometimes reported to be characteristic of mankind, yet accomplish a thing which is not only impossible at present for a man to do, but also very difficult indeed to understand. The spider crab, *Inachus mauritanicus*,¹ is very fre-

¹ By an unfortunate error in nomenclature *I. mauritanicus* (Lucas) was called *I. scorpio* (Fabr.) throughout my monograph.

quently infected with a species of rhizocephalous Cirripede called *Sacculina neglecta* (5). This parasite at first lives a free existence as a minute larva; it then fixes itself to a hair on the outside of its host and passes into the body of the latter a small group of cells which find their way to the blood-space round the intestine. Here they begin to grow very rapidly into a branched tumour-like body which sends its ramifications into every part of the body-cavity of the crab. A certain part of the tumour becomes applied to the ventral body-wall of the crab at the junction of thorax and abdomen, and at this point the reproductive organs, etc., of the adult *Sacculina* are developed and finally thrust to the outside in a muscular bag which remains attached to the crab and swells to a large size, gaining its nutriment from the system of branching roots which continue to multiply and grow inside the crab's body.

Now the chief effect which the parasite exerts on the crab is to cause the complete or partial atrophy of the internal generative organs, with their ducts, while remarkable changes take place in the structure of the external secondary sexual characters. Of 1000 specimens of *Inachus* infected with *Sacculina* examined by me at Naples, 70 per cent. of both males and females showed very distinct alteration in their secondary sexual characters, while all showed some degree of reduction or atrophy of the gonad. Of the many thousands, at present well over 5000 specimens, of uninfected *Inachus* examined, only one specimen showed any trace of the changes such as were met with in the infected individuals. This specimen, which was a perfect hermaphrodite both externally and internally, may have been an instance, such as occurs with extreme rarity in decapod Crustacea, of hermaphroditism apart from parasitic castration, but it is equally possible that it was really a crab that had recovered from an infection with *Sacculina*, and had undergone several moults so as to lose the scar characteristic of crabs that have been once infected. I mention these facts with regard to the numbers of specimens examined, because it is important to realise not only the

extent of the material upon which my conclusions are based, but also the invariable certainty and regularity of the effect observed.

The sexes of normal uninfected *Inachus mauritanicus* differ in that the adult male (Pl. 30, figs. 1 and 2) possesses greatly elongated and swollen chelæ, while the abdomen is small in size, is carried flatly opposed to the thorax, and is furnished with only two pairs of appendages—viz. a large pair of stout copulatory styles and a greatly reduced pair of appendages behind them. The adult female (Pl. 30, figs. 10 and 11) has small, slender chelæ and an exceedingly broad, trough-shaped abdomen which is furnished with four pairs of bi-ramous appendages. These appendages are clothed with long hairs, some of which are used for attaching the eggs.

The sexual difference in the chelæ is not developed until maturity, but the differences in the abdomen are marked soon after the Megalopa larval stage and long before maturity. The female, however, at first goes through a stage in which the abdomen is comparatively small and flat, and the appendages are short and rod-like without the filamentous hairs characteristic of the adult (Pl. 30, figs. 13 and 14).

The males infected with *Sacculina* show every degree of modification towards the female type (Pl. 30, figs. 3 to 9). In some the only change to be observed externally is the reduction in size of the chelæ, and perhaps a slightly tapering form induced on the usually stout and blunt copulatory style (Pl. 30, fig. 5). In others the abdomen is somewhat broadened (Pl. 30, figs. 3 and 4), and in a further stage the abdomen is distinctly broadened and somewhat trough-shaped, while perhaps one or two additional appendages are developed in a rudimentary condition behind the reduced copulatory styles (Pl. 30, fig. 6). If such forms are carefully dissected the gonad is observed to be greatly shrivelled, but it can still be clearly recognised as a testis, while a few clumps of spermatozoa may be found in the vasa deferentia. Finally we obtain forms (Pl. 30, figs. 7, 8 and 9), usually

among the smaller and medium-sized individuals, in which the chelæ and abdomen have taken on the complete adult female appearance, the only male character remaining being the copulatory style, which is sometimes reduced to a minute knob (fig. 8). I was for a long time in doubt as to which sex these highly modified crabs belonged, as the majority of them on dissection proved to possess no remains of the gonad and gonoducts, except in certain cases some small shreds of germinal epithelium. In a few, however, the remains of the gonoducts were found, and in all cases they were in the position of the vasa deferentia, proving the animals to be males in which the modification towards the female type had gone very far. Further reflection showed that all these highly modified crabs were originally males, and this was proved without doubt by their invariable possession of the copulatory style in either a complete or greatly aborted state. For in the case of the females, although they, too, undergo characteristic changes, they never make any approach either in the chelæ or abdomen towards the male state, and there is never any trace in them of the development of the copulatory style.

It is therefore altogether impossible that other crabs, in which the sex cannot be determined by the internal gonad or its ducts, should be females which, contrary to all experience, had suddenly developed the single male character of the copulatory styles. That they were originally males, however, is shown by the perfect gradational series, which can be traced from hardly modified males up to those specimens in which the only male character retained is the presence of the copulatory styles.

It is important to notice that the males, when they develop the female characters to any great extent, invariably exhibit these characters in the form in which they occur in the adult female, the abdomen assuming the trough-like appearance, and the appendages being slender and provided with filamentous hairs. I emphasise this point because certain people have argued with me that the modification of the male was

not due to the assumption of definite female characters, but a "reversion" to an "undifferentiated ancestral condition," or to "an embryonic state." But if this were so, the male should at any rate assume the comparatively undifferentiated state which is actually passed through by the young immediately after the megalopa stage, and which is retained by the female until the first brood of eggs is produced, viz. the small, flat, plate-like abdomen and the rod-like form of the appendages. Now, as a matter of fact this form is never assumed by the male as the result of parasitic castration, the female characters being acquired, if imperfectly, yet with the definite characters only found in adult females which have produced a brood of eggs. This fact alone seems to me to demolish the above-mentioned argument, but it is even more completely answered by the fact, soon to be described, that certain of these male specimens may, on recovery from the disease, actually produce ova as well as spermatozoa in their regenerated gonads, thus proving that they actually have developed true female characters and have not merely returned to an undifferentiated condition of an altogether supposititious nature. The infected females, as we have already stated, do not show in any character any approach towards the male secondary sexual characters, though dissection proves that in all cases the ovary is arrested in development, or even completely aborted. The only secondary sexual character affected is the condition of the abdominal appendages, which may be greatly reduced in size (Pl. 30, fig. 12). There is never any approach to the male either in the chelæ, or in the shape of the abdomen, or in the development of an appendage corresponding to the copulatory style of the male.

We have now to consider the case of the highly modified males which have developed the external female characters but retain the copulatory styles. We have seen that in all these specimens the gonad is reduced to a few shreds of undifferentiated germinal epithelium, and in some cases the remains of the vasa deferentia. In a very few cases such

crabs were found in a state of nature to have recovered from the disease, the *Sacculina* having dropped off and left the characteristic circular scar on the abdomen where it was previously attached. Of a large number of crabs experimentally freed of the parasite a few also survived for several months. Of these specimens, three which had recovered naturally and one which had been experimented on, were found to have regenerated the gonad, which had grown to a considerable size. The gonad was found to contain a certain amount of adult spermatozoa and a number of ova, some of them small and immature, others of a very large size and distended with the reddish-coloured yolk, which normally appears in the eggs as they approach maturity.

The fact that the alteration of the male, under the influence of the parasite, is consummated by the final assumption of complete internal as well as external hermaphroditism is, I believe, without parallel, and confers on this case a peculiar definiteness and value which we cannot obtain elsewhere. The phenomenon appeared to me so strange and so little likely to gain credit from people who had not actually investigated the matter, that I was greatly pleased when Mr. F. A. Potts undertook, at my suggestion, to examine the matter in a parallel instance, namely the effect of the parasite *Peltogaster* on the hermit-crab, *Eupagurus* (15). The investigation of this case offered considerably more difficulty than the case I had examined, but he was able to obtain a series of results which happily place the main conclusions outlined above on a very sure footing. Stated concisely he found that, as in *Inachus*, so in *Eupagurus* the infected males assumed to varying degrees of perfection the female characteristics,¹ but that the females, as in *Inachus*, never acquired any male characters, although they might show reduction of their own secondary sexual characters. The most remarkable result, however, obtained by him consists in the discovery that in a very large number of modified males, while the

¹ The female characters assumed by the male are here, as in *Inachus*, those of the adult breeding female.

parasite was still on them, small ova were developed in the testes. This observation, while differing in an interesting manner from what occurs in *Inachus*, where the ova are not found until after recovery, yet confirms the account I have given for *Inachus* in a very convincing way.

If we consider the facts related above in their bearing on the problem of the correlation of the primary and secondary sexual characters, it is evident that we are provided with some instructive evidence. In the first place we observe the male developing the secondary sexual characters of the female, and this it does, not merely in a negative manner by returning to some intermediate, indifferent condition, as usually happens in the case of ordinary castration, but by positively acquiring characters which normally only appear in the adult breeding female. Now, we may hold two opinions with regard to these males—either that their resemblance to the female is a spurious one, and that the development of the female secondary sexual characters is due in them to a different cause to that which conditions their development in the female, or else it is a true resemblance due to the same cause. That the latter alternative is correct is shown by the fact that these males may subsequently develop typical ova, because we cannot require more of an animal to prove its female nature than that it should produce ova and exhibit all the secondary characters of the female as well. The infected males, therefore, develop the female secondary sexual characters for the same reason that the female does. Now what is this reason in the female? In the female the development of the secondary sexual characters is correlated with that of the ovary. Thus the adult form of the abdomen and the form of the abdominal appendages is not assumed until the ovary is ripe, while the atrophy of the ovary, as a result of the presence of *Sacculina*, causes the atrophy to some degree of the appendages. In the case of the female, therefore, we might assume that the ovary produces a substance or internal secretion which causes the development of the secondary sexual characters.

But this cannot possibly apply to the infected males, because they develop the same female secondary sexual characters before there is any ovary present at all, much less a mature ovary ready to produce ripe eggs. Now these males, although they develop the female secondary sexual characters when there is no ovary present, yet subsequently they may regenerate an ovary from the shreds of germinal epithelium remaining from the degenerated testis. In other words they have the potentiality to produce an ovary, and we may safely argue that it is this potentiality which enables them to produce the secondary sexual characters before the actual ovary is there.

In this conception it appears to me lies the solution of the uncertain nature of the correlation existing between primary and secondary sexual characters in general. It is not necessarily the presence of a differentiated gonad producing some internal secretion which causes the development of the corresponding secondary sexual characters, but it is the potentiality to form that gonad. Thus the development of the secondary sexual characters is not primarily dependent on the gonad, but the development of both is dependent on a third factor. If we attempt to formulate what this factor actually is, it appears to me legitimate to represent it as the presence of a substance having the nature of an internal secretion, which circulates through the body and controls the differentiation of the primary and secondary sexual characters. I have called this hypothetical substance the "sexual formative substance," and we must suppose that two kinds of it exist, male and female. By this theory we can account for the imperfect nature of the correlation between primary and secondary sexual characters, and also for the development of the female secondary sexual characters in infected male crabs before the development of an ovary, which is unaccountable on the theory that the ovary produces the substance necessary for the development of the secondary characters.

It is, however, a notorious fact that the mere removal of

the gonad in the great majority of animals directly inhibits the full development of the secondary characters, and it may appear that the theory outlined above gives no explanation of this fact. I clearly realised this in my first statement of the theory, and put forward the suggestion that the sexual formative substance accumulated, especially at maturity, in the gonad, and that the removal of the gonad removed a large quantity of the substance and so inhibited the growth of the secondary sexual characters. I do not feel, however, that this explanation is at all adequate, principally for the reason that the removal of the gonad in the young immature animal has usually a more pronounced effect than its removal in the adult. It is therefore more probable that the sexual formative substance is in many cases actually worked up and qualitatively altered by the gonad, and that its presence in this altered state is essential in most cases for the full development of all the sexual characters.

We may indeed hold, with the highest degree of probability, that the sexual formative substance, both male and female, is by no means a single simple substance, but that it consists of numerous substances continually changing during development, and both acting and acted on by the various organs of the body. A view very similar to this is held by Mr. Walter Heape, as the result of his experiments (16). He considers that there is present a "generative ferment" which is produced somewhere in the body and which governs the activity of the generative glands, and another substance, "gonadin," secreted by ovary or testis, which controls the other sexual characters, but he is clearly of opinion that in certain cases it may be the generative ferment which controls the secondary sexual characters, and this would bring his view into close agreement with my own.

The theory which has been outlined above, and which differs from other theories chiefly in that it attempts to include those cases in which the correlation between primary and secondary sexual characters is of an uncertain and perplexing nature, has been attacked by Mr. Cunningham (14)

who regards my views as illogical, self-contradictory, and inconsistent with the state of modern biology; indeed, he lays so many charges to my account that modesty compels me to suspect that some of them may be true; but I can hardly think that the difference between our respective views is proportional to the severity of his indictment. The only difference of importance which I can discover is that, whereas he believes that the internal secretion controlling the development of the secondary sexual characters is always produced by the differentiated gonad, I do not believe that this theory covers all the essential facts, but that we must assume some common factor at the back of both primary and secondary characters which may act to a certain extent on either separately, in an independent manner. On the other hand, I have never denied the direct influence of the primary character on the secondary to a limited extent, as it appears to me to be clearly proved by a very large body of facts.

In conclusion, Mr. Cunningham agrees with me in believing that the explanation of the development of the secondary sexual characters, and of their correlation with the primary, depends on the presence of a substance, sexual formative substance or internal secretion, circulating in the body, which in some manner activates the cells of various organs and parts of the body and causes them to develop and to become differentiated according to sex, and I think that he would agree with me that a great deal of experiment and observation is necessary before we can decide with any certainty as to the nature of this substance or substances.

That we are dealing with the presence of an internal secretion is strongly suggested by the analogy of the internal secretions produced by other organs, such as the thyroid and other ductless glands. In the development of the sexual characters we perceive distant parts of the body being affected in a parallel manner at the same time, while a removal of part of the system may profoundly modify other distant parts. This inter-connection can only be accounted for in one of two ways—either by the supposition that it is due to

nervous communication, or else by means of substances conveyed in the blood or body-fluids. The former supposition is ruled out by a number of experiments, such as the severance of the nerves to the reproductive organs, etc., so that we are perforce thrown back on the second supposition of internal secretions, although the participation of the nervous system is not altogether precluded.

It would also seem probable that sexual differentiation does not solely depend on the presence and nature of these substances, but rather in the interaction of these substances with the cells of the organism, which may themselves be differentiated beforehand in the two sexes. The attempt to analyse the nature of the sexual formative substance and its relation to the primary and secondary characters will occupy us in succeeding parts.

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EXPLANATION OF PLATE 30,

Illustrating Mr. Geoffrey Smith's paper on "Studies in the Experimental Analysis of Sex."

In figs. 1, 3, 4, 7, 10 and 13 only the right chela is shown, the other thoracic limbs being omitted.

All figures are of *Inachus mauritanicus*.

Fig. 1.—Adult normal male.

Fig. 2.—Under-side of abdomen of normal adult male.

Fig. 3.—Male infected with *Sacculina*, showing reduction of chela and slight broadening of abdomen.

Fig. 4.—Male infected with *Sacculina*, showing reduction of chela and increased broadening of abdomen.

Fig. 5.—Under-side of abdomen of fig. 4, showing attenuated copulatory styles and slight hollowing-out of abdomen.

Fig. 6.—Under-side of abdomen of a similar male specimen, showing reduction of copulatory styles and presence of asymmetrically placed swimmerets, characteristic of female.

Fig. 7.—Male infected with *Sacculina*, which has assumed complete female appearance.

Fig. 8.—Under-side of abdomen of fig. 7, showing much reduced copulatory styles and reduced swimmerets.

Fig. 9.—Under-side of abdomen of a similar male specimen, showing well-developed copulatory styles and swimmerets.

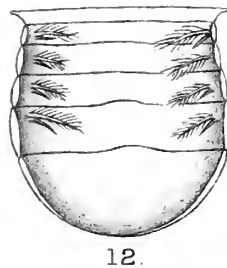
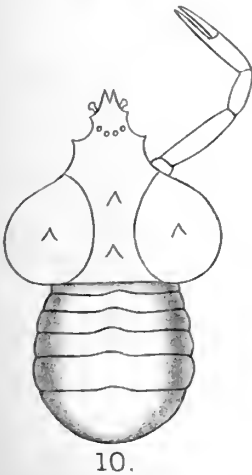
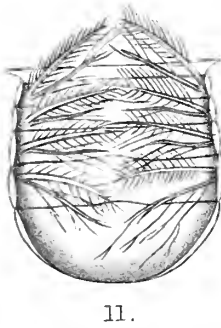
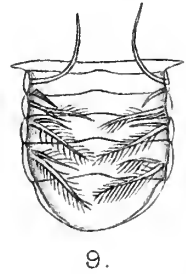
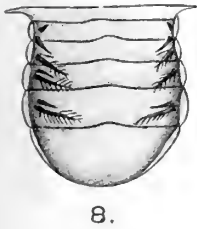
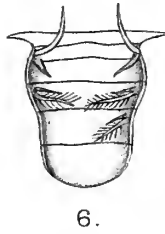
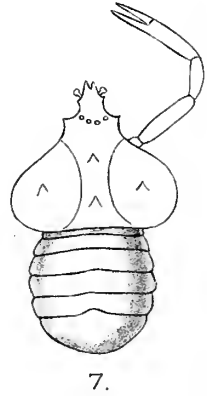
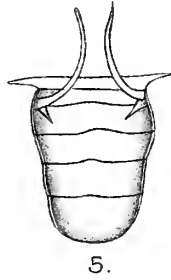
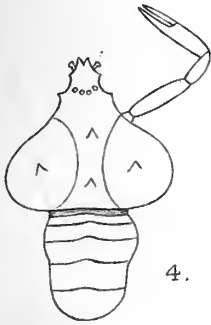
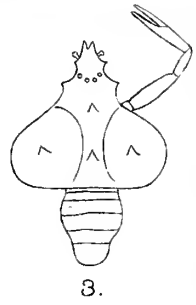
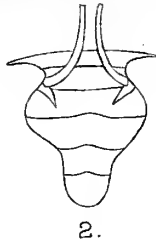
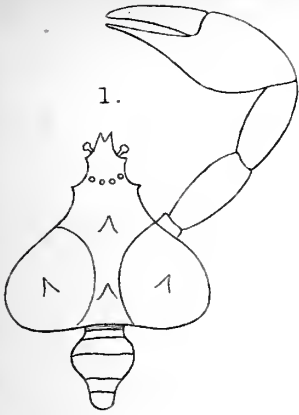
Fig. 10.—Adult female, normal.

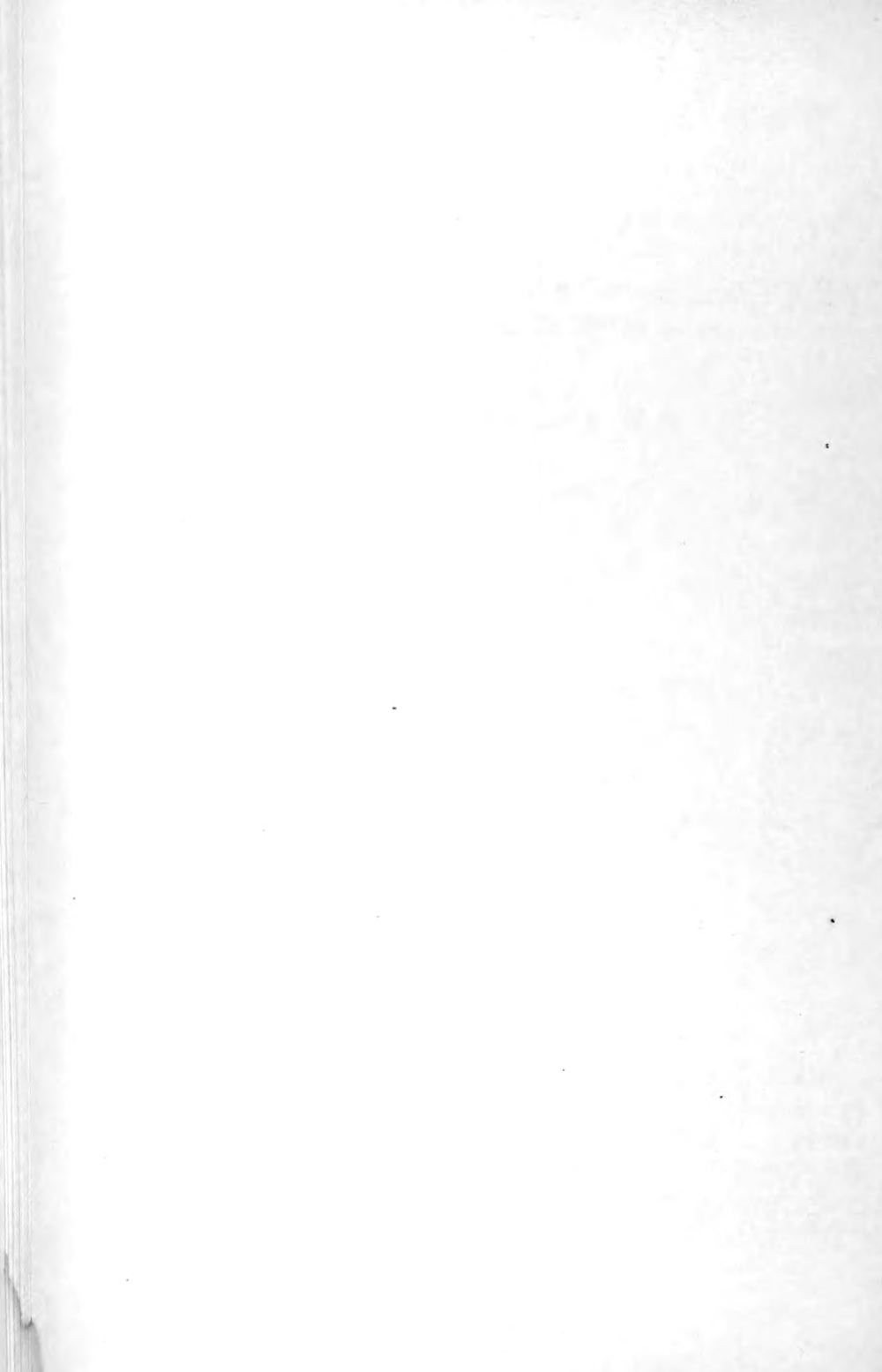
Fig. 11.—Under-side of abdomen of fig. 10, showing swimmerets and trough-shaped abdomen.

Fig. 12.—Under-side of abdomen of female infected with *Sacculina*, showing reduction of swimmerets.

Fig. 13.—Immature female, showing small flat abdomen.

Fig. 14.—Under-side of abdomen of fig. 13, showing flat surface and rod-like swimmerets.





Some Points in the Physiology of Lamellibranch Blood-Corpuscles.

By

G. H. Drew, B.A.Cantab

With Plate 31.

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INTRODUCTION.

THE investigations described in this paper were carried out on the blood of *Cardium norvegicum*. This animal was chosen as a type as it presents several features which render it especially suitable for hæmatological work. Among these may be mentioned the ease with which the blood can be obtained in large quantities, the relatively large size of the

corpuscles, the great vitality of the animal, and its large and readily protruded foot.

One object of the present work was to investigate the "clotting" of Lamellibranch blood-corpuscles, and to follow the relation this process bears to the natural cessation of hæmorrhage from a wound and its subsequent healing. Another object was to investigate the phagocytic action of the corpuscles on bacteria, and to find whether they showed any chemiotactic action towards cultures of bacteria or extracts of dead tissues.

My results show that some change takes place in the corpuscles when the blood is shed, which causes them to agglutinate round the edges of a wound, and that these masses of corpuscles are connected by thin protoplasmic processes running across the wound. These processes thicken and contract, and so draw together the edges of the wound. There is some evidence to show that the change in the corpuscles, which makes them agglutinate, is produced by a contact stimulus imparted on contact with a foreign body or with injured tissues. Some of the corpuscles have a phagocytic action on bacteria, and show a positive chemiotactic attraction towards cultures of bacteria and extracts of dead tissues, so that their protective function appears to be the same as that of the leucocytes of mammalian blood.

My thanks are due to the Marine Biological Association of the United Kingdom for their kindness in granting me a table at their Plymouth Laboratory.

HISTORICAL.

The chemistry of the blood of the Lamellibranchiata has been thoroughly investigated by Ray Lankester (9, 10, 11, and 12), Cuénot (2), Griffiths (8), and others, but a number of observers have described the organised elements of the blood with very varying results. Their conclusions are summarised by de Bruyne (3). He himself recognises seven varieties of corpuscles in Lamellibranch blood, taking as his types

Mytilus edulis, *Ostrea edulis*, *Unio pictorum*, and *Anodonta cygnea*. Cuénot (2) describes a "glande lymphatique" at the base of the gills, where the corpuscles originate, and recognises coarsely and finely granular corpuscles, and a third variety consisting of a nucleus with very little surrounding protoplasm. He considers that these are all derived by degenerative changes from the coarsely granular form. In the case of *Cardium norvegicum* I agree with Cuénot's classification, but am inclined to consider that he has scarcely published sufficient evidence to warrant the statement that the other varieties are degenerated forms of the coarsely granular corpuscles.

The fact that the plasma of Lamellibranch blood does not coagulate has long been known, and Geddes (7), in 1879, described the plasmodial masses formed by the agglutination of the corpuscles in shed blood. Dakin (4), in his monograph on *Pecten*, suggests that these plasmodia may act as plugs by which hæmorrhage from a wound may be checked, but does not enter into the causes which determine this agglutination, nor its function in the healing of wounds.

The question of phagocytosis has not been fully investigated. Ray Lankester (11 and 12) first recognised the phagocytic action of certain green amœboid cells which he found on the surface of the gills of "green" oysters, and later de Bruyne (3) described the way in which certain wandering corpuscles invade and destroy the epithelium of the gills, and finally escape. De Bruyne attributes an excretory function to these cells, and considers the "aqui poriferi," which were formerly supposed to communicate directly between the blood-spaces and the surrounding water, to be due to erosions of the epithelium caused by the emigration of these cells. In the same paper he mentions the ingestion of carmine granules by the phagocytes. No account has yet been published of the phagocytic action of the corpuscles on bacteria, though, owing to the fact that the blood as a whole does not coagulate, many difficulties in the way of such investigations are eliminated.

METHODS.

Collection and Preservation of Living Animals.

Cardium norvegicum can be obtained in the neighbourhood of Plymouth by dredging on several grounds at a depth of twenty to thirty fathoms. It was formerly quite plentiful, but the supply has been falling off somewhat during the last few years. The animal will live for many hours out of water, and for some days if packed in damp seaweed. I have kept a number for months in the Laboratory in basins into which a small jet of sea-water flowed. Sufficient food is obtained from the minute forms of life present in the water circulating in the experimental tanks, and any artificial method of aëration is unnecessary. The vitality of the animal is so great that a relatively large volume of blood may be withdrawn, and complete recovery ensue, in the course of a few days, after which the blood appears to be normal in constituents and quantity.

Collection of Blood.

The blood can be most conveniently obtained from the anterior adductor muscle. When the valves of the shell are slightly apart, a small wedge is introduced between them to prevent closing of the shell; this usually causes the protrusion of the large and powerful foot, which is violently waved about, and may displace the wedge unless it has been inserted near the anterior adductor muscle, where it is beyond reach of the foot. A clean, fine-pointed glass pipette, fitted with a rubber teat, is then introduced between the fibres of the adductor muscle, and the blood slowly withdrawn.

The following precautions should be taken:

The pipette should not be forced through the adductor muscle so as to rupture any of the viscera; it should penetrate about half-way through the muscle, and then be slightly withdrawn to free the end.

A pipette with a jagged or sharp point should not be used, as this tends to cause agglutination of the corpuscles.

By this method I have obtained as much as 20 c.c. of blood from a large specimen of *Cardium norvegicum*.

Fixing and Staining Methods.

By far the most satisfactory results were obtained by simple fixation with corrosive sublimate, and staining with aqueous eosin and methylene blue.

A drop of blood is allowed to fall on a slide from the collecting pipette, and is then left in a moist chamber for from half an hour to an hour to allow the corpuscles to expand. Two or three drops of a saturated solution of corrosive sublimate in sea-water should then be added, and left for five minutes. This is drained off, and the slide washed in 90 per cent. alcohol. After this the slides may be placed in water for a few minutes, and then stained for some time in a dilute aqueous solution of eosin; this is washed off, and Loeffler's methylene blue, diluted 1 in 100, is added, and left for from one to two minutes. The slide is finally washed in distilled water, allowed to dry (not blotted), and mounted in xylol balsam.

Almost equally good results were obtained by fixation with osmic acid, but no other fixatives employed were really satisfactory. No variety of the Romanowsky stain gave such good results as treatment with eosin and methylene blue, one after the other. Hæmatoxylin stained the nuclei well, but interfered with the differentiation of the eosinophil granules.

Treatment of the fresh blood with 1 per cent. acetic acid and methylene green differentiates the nuclei of the corpuscles, but fixation is not sufficiently rapid to enable the corpuscles to be examined in the expanded condition.

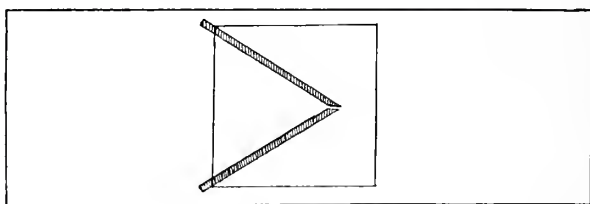
Sections of the tissues showing wounds, etc., were fixed in corrosive sublimate, and stained with Ehrlich's hæmatoxylin and erythrosin, or van Gieson's stain.

Methods of Observing the Process of Agglutination of the Corpuscles.

The plasmodial masses, formed by agglutination of the corpuscles, can be produced by shaking the blood in a tube, or by stirring a drop on a slide with a needle, and can then be directly examined under the microscope.

To study the exact process by which they were formed I employed the following method :

Two extremely thin strips of plasticine were placed on a slide, inclined to each other at about an angle of 45° , and arranged so as to leave a very narrow opening between their convergent ends. A cover-slip was applied, and pressed firmly down, so as to prevent the escape of liquid under the bands



of plasticine. A drop of blood can then be run under the cover-slip, and can only escape from the cell at the narrow end, though which it may be drawn with a piece of filter paper. In some experiments a few strands of cotton-wool, or glass-wool, were placed in the narrow opening, the fibres being arranged as far as possible parallel to one another and in the direction of the flow of the blood. Agglutination of the corpuscles occurs readily as the blood passes through the narrow end of the cell, and the process can be observed under the microscope.

The way in which these agglutinated masses of corpuscles close the opening of a wound, and so stop hæmorrhage, was studied by making small incisions in the foot when extended, and then fixing by immersing the whole foot in saturated corrosive sublimate, and finally cutting serial sections.

Where the healing of a wound in later stages was followed, the animal was narcotised with the foot in an extended condition by a 1 per cent. solution of cocaine in sea-water; the required portion of the foot was then cut off, and fixed, and sectionised as before. In these experiments it is advisable to keep the animals in separate basins with a good flow of water, as if overcrowded, a number die, presumably from infection through the wound.

Methods of Studying Phagocytosis.

The bacteria used for this purpose were obtained by inoculating peptonised fish-broth, made with sea-water, with a platinum loop which had been passed along the edge of the mantle. This resulted in a mixed culture of bacteria, in which a rather large non-motile bacillus with rounded ends, and a tendency to form diplo-bacilli, much predominated.

The actual process of phagocytosis was observed by adding a loopful of the diluted culture to a drop of blood. Permanent slides, showing ingested bacteria, were prepared by leaving a drop of blood, to which bacteria had been added, for from one to two hours in a moist chamber, and then fixing and staining by the same method as that employed in the preparation of stained blood-films.

Further observations were made by filling thin-walled capillary tubes with cultures of bacteria, sealing them at one end, and introducing the open end into a drop of blood on a slide. A cover-glass with a small quantity of wax at each corner was then placed on the drop, and the tube containing the bacteria kept under observation.

Similar experiments were conducted by introducing capillary tubes containing cultures, etc., into the adductor muscle of the animal, which may be replaced in the water and left for some hours. The tubes were then withdrawn, and the number of corpuscles which had entered the tubes were noted under the microscope.

In these experiments the capillary tubes should be marked

with a diamond, broken clean across, and passed through the flame, so as to obviate jagged ends, which tend to cause an agglutination of the corpuscles around the end of the tube.

DESCRIPTION OF THE BLOOD.

The blood of *Cardium norvegicum* is a somewhat opalescent fluid, appearing slightly yellow by transmitted, and blue by reflected, light. On shaking, the corpuscles stick together, forming small white floccular masses which fall to the bottom of the liquid.

The plasma can be obtained free from the corpuscles by filtering, and appears of the same colour as the blood. It contains hæmocyanin (Cuénot) (2). Its reaction to litmus is neutral. On heating to 74° C. it becomes distinctly cloudy, and in time a floccular precipitate of coagulated proteid is produced, which is soluble in alkalies and acids, but is reprecipitated on neutralisation. The addition of an equal volume of 90 per cent. alcohol produces a white precipitate. The plasma gives the usual proteid reactions, such as the xanthoproteic and Biuret reactions, a brick-red precipitate with Millon's reagent, a white ring on the addition of nitric acid (which does not disappear on warming), and a white precipitate with potassium ferrocyanide and acetic acid.

An analysis of the blood for salinity, by titration with silver nitrate, using potassium chromate as an indicator, gave a chlorine value very slightly higher than that of the seawater in which the animal was living. This small increase was probably due to evaporation while collecting the blood, boiling to remove the hæmocyanin, and filtering. Without very specialised apparatus it is almost impossible to go through these operations without a small loss due to evaporation.

Cuénot has estimated the total salts in the blood of a number of marine invertebrates living in waters of various salinities. He evaporates and incinerates the blood, and estimates the salts gravimetrically. By this method he

invariably found that the total salts in the blood was slightly less than that in the water in which the animal lived, but his published researches do not extend to any species of *Cardium*.

The organised elements in the blood of *Cardium norvegicum* consist entirely of amœbocytes. As seen in the freshly drawn blood, they appear as slightly granular, colourless corpuscles showing a number of short pseudopodia; they vary considerably in size, but the different varieties of the corpuscles cannot be easily distinguished in unstained preparations, nor can the nucleus be clearly made out.

If a slide in which the cover-glass is supported at the corners with wax, so as to ensure a fairly thick film of blood, be kept under observation, it will be noticed that the corpuscles perform slow amœboid movements. After the lapse of about half an hour many of the corpuscles can be seen fully extended, and may then measure three or four times their diameter in the contracted state. In this condition they are very thin and transparent, and can be most conveniently observed under a narrow cone of illumination. The pseudopodia are often remarkably long and slender.

Stained preparations show that the corpuscles can be divided into three classes:

(1) Finely granular eosinophil corpuscles (fig. 1). These are the largest corpuscles present. They possess a single round or oval nucleus, and a relatively large amount of protoplasm, which contains a number of extremely small eosinophil granules, chiefly concentrated round the nucleus. The longer pseudopodia are as a rule free from these granules at their extremities, but there is usually a line extending round the periphery of the corpuscle, which stains slightly with eosin.

(2) Coarsely granular eosinophil corpuscles (fig. 2), similar to the finely granular variety, but slightly smaller, and possessing large, well-defined, eosinophil granules.

(3) Basophil corpuscles (fig. 3). These are much smaller than the two preceding varieties, and do not take on the eosin stain at all. They possess a single round nucleus, and a very small amount of protoplasm, which also takes on the

blue stain. In fresh unstained preparations they can often be distinguished by their small size, almost spherical shape, and somewhat high power of refraction.

Differential counts gave the following as the average relative proportion of the corpuscles :

Finely granular eosinophil	48 per cent.
Coarsely granular eosinophil	44 „
Basophil	8 „

THE PROCESS OF AGGLUTINATION OF THE CORPUSCLES, AND ITS RELATION TO THE HEALING OF WOUNDS.

The small white floccular masses produced when the freshly drawn blood is shaken in a tube, consist of a number of corpuscles which have coalesced to form a compact mass ; the individual corpuscles are still distinguishable, and those round the periphery of the mass protrude pseudopodia. This coalescence of the corpuscles also occurs when the fresh blood is stirred with any foreign body, or when the corpuscles come into contact with any rough surface. If the blood be carefully collected by a pipette with well-rounded ends, and dropped on a slide, there will be comparatively little agglutination of the corpuscles, but if the pipette be at all dirty, so as to present a rough surface to the blood, or if the end of the pipette be at all jagged, there will be considerable coalescence.

When the blood is allowed to flow through a meshwork of cotton-wool, nearly all the corpuscles will stick to the strands and form dense agglutinated masses; if glass-wool be employed instead of cotton-wool, this result is not so marked.

It seems possible that this power of agglutination depends on some change produced in the corpuscle by the stimulus of contact or friction with a non-living body.

This agglutination can be studied more fully in the plastic cell already described. If the blood be drawn through the small opening, a clot of corpuscles will soon be formed, which will close the opening, and so prevent further escape

of blood. Individual corpuscles can be watched as they near the opening, and it will be seen that the majority touch the bands of plasticine at least once before they adhere to its surface. When the stream of blood is extremely slow, so that the momentum of the corpuscles on contact is slight, a corpuscle may touch any foreign body three or four times before adhering, but if the momentum of the corpuscle be greater, it may adhere on the first or second contact. In the case of contact with a polished surface, such as glass, the power of agglutination is not so marked. Once a corpuscle has adhered, it possesses the power of sticking to any other corpuscles that may come in contact with it, and these, in their turn, can then adhere to others. That this power of mutual adhesion is not possessed by the corpuscles in the freshly drawn blood, is proved by the fact that corpuscles may often be seen impinging without showing any tendency to adhere, until one of them touches some foreign body, or meets with other agglutinated corpuscles. Two corpuscles, one or both of which have developed this power of agglutination, may frequently adhere to each other by their pseudopodia, and then become separated by the blood-current; when this occurs, a thin protoplasmic connection seems always to remain between the corpuscles.

This phenomenon can best be studied by placing a few strands of cotton-wool between the convergent bands of plasticine. In this case, the corpuscles can be seen to form agglutinated masses along the strands of cotton-wool, and frequently thin protoplasmic connections are visible between masses of corpuscles situated on different strands of cotton (fig. 4). At first these bands may be so thin as to present considerable difficulty in resolution, unless oblique illumination be employed. Even when invisible, their presence is often shown by their power of arresting and adhering to passing corpuscles. I have frequently followed the course of two corpuscles, which had adhered by their pseudopodia, and then become separated in the blood-stream, and settled down on neighbouring strands of cotton-wool. In such cases a

thin protoplasmic connection, often of remarkable length relative to the size of the corpuscles, was either directly visible, or was demonstrated by the adhesion of passing corpuscles.

If two agglutinated masses of corpuscles, connected by one or more such protoplasmic strands, be kept under observation for several hours, it will be seen that the strands slowly thicken. Corpuscles from each end may travel up a strand and appear to become merged in it, as do also any free corpuscles which may have adhered to it. The corpuscles at each end also send out pseudopodia along the strand, and may be drawn up into it as it thickens.

During this process the strand contracts, increasing proportionately in breadth. The force of this contraction is often sufficient to draw together the two neighbouring fibres of cotton to which the corpuscles have adhered, and by this means the two original masses of agglutinated corpuscles may finally be fused into one.

This phenomenon was studied as far as possible in life by making small incised wounds in the foot when under water, fixing after varying intervals, and sectionising. Unfortunately the extremely delicate protoplasmic strands, formed between adjacent masses of corpuscles in the earliest stages of agglutination, did not withstand the fixing and embedding process, but sections of a wound, that had been left for from one to two hours before fixation, showed agglutinated masses of leucocytes on the edges of the wound, with connecting bands running in all directions, thus forming a plug which would at least prevent the escape of corpuscles through the wound, and probably much hinder the escape of the plasma. Sections of wounds a few hours older showed the wound completely blocked by the agglutinated corpuscles. Sections of still older wounds showed that the process of healing in many ways resembled that in Mammalia. The agglutinated masses of corpuscles soon become more or less structureless, and much resemble a mass of fibrin. This is then invaded by other corpuscles which have a phagocytic action, and

they are accompanied by connective-tissue corpuscles with elongated nuclei, which form connective tissue. At the same time, in the case of small incised wounds of the foot, the cut muscle-fibres grow across, and the surface epithelium joins up over the surface of the wound, so that after the lapse of about three weeks the site of the wound may be almost indistinguishable in sections.

I consider that the process as observed in the plasticine cell is probably identical with that in Nature. When a wound is made, the tissues along the immediate margins of the wound have their vitality impaired, and though perhaps not dead, are at least in an abnormal state. As the blood escapes, the corpuscles impinge on these tissues and agglutinate, and connecting strands of protoplasm may be formed between masses of corpuscles on opposite sides of the wound, in the same manner as that described when the blood is drawn through cotton-wool. The subsequent thickening and contraction of these bands of protoplasm would tend to draw the edges of the wound together, and cause complete fusion between the neighbouring masses of agglutinated corpuscles.

It is obvious that some change takes place in the corpuscles of blood which has been withdrawn from the animal, which confers on them the power of agglutination. Of the nature of this change I have no evidence. Arguing by analogy, it seems possible that it is due to the liberation of some enzyme from the corpuscle. There is no visible change in the contents of the cells after agglutination, and stained preparations show that both the large and small eosinophil granules are still present.

The effect of exposure to air, as a predisposing cause of agglutination, may be eliminated by the fact that a small wound, made under water, soon becomes plugged with agglutinated corpuscles, and also by the fact that agglutination occurs when the blood has been collected under water in a narrow pipette already partially full of sea-water. This does not dispose of the action of dissolved air, but it is reasonable to assume that the blood during its passage through the gills

contains approximately the same amount of dissolved air as the surrounding water.

The corpuscles also agglutinate in the case when the animal, with the shell-valves wedged open, is washed with distilled water, and the blood withdrawn with a dry pipette. This disposes of the possibility of agglutination being caused by admixture with sea-water. Mixture of the blood with hypertonic or hypotonic salt solutions also does not hinder agglutination.

The possibility that some stimulus, conveyed to the corpuscle by contact or friction with some foreign body, is the predisposing cause of agglutination, is suggested by the fact that corpuscles can be seen to agglutinate after contact with a foreign body, and more especially by the fact that the rapidity of the change in the corpuscle appears to depend on its momentum when impinging on the body. The fact that agglutination does not occur so readily after contact with a polished surface, such as glass, where there would be less friction, is also in favour of this theory.

PHAGOCYTOSIS.

The phagocytic action of the corpuscles on bacteria can be watched by placing a drop of blood on a slide, and adding a loopful of a culture of bacteria in broth diluted with sea-water. The corpuscles can be seen to send out pseudopodia in the direction of the bacteria, and engulf them. They may then be fixed and stained as before (figs. 5 and 6). Agglutinated corpuscles do not appear to possess this power, but motile bacteria, in the course of their movements, may touch and adhere to them; this is probably a purely passive action on the part of the corpuscles. No phagocytic action on the part of the basophil corpuscles was observed, nor did stained preparations show that this had taken place. Experiments were tried by introducing capillary tubes containing cultures into a drop of blood under a cover-slip, supported at the corners by wax; in this case a certain number of corpuscles

could usually be seen to enter the tube, and there was an apparent concentration of the corpuscles about the mouth of the tube after the lapse of from half an hour to an hour, but though negative results were given in check experiments, in which capillary tubes filled with sea-water were employed, yet the number of corpuscles were usually so few (four to eight on an average in half an hour), that the results cannot be considered conclusive. Much more conclusive results were given by introducing similar capillary tubes into the anterior adductor muscle, and leaving them there about two hours, having inserted a small wedge between the valves of the shell to prevent the breaking of the tubes. Tubes filled with the following fluids were employed, and all introduced at the same time into the same animal:

(1) Culture of non-motile bacilli in peptonised fish-broth made with sea-water.

(2) Peptonised fish-broth made with sea-water.

(3) Culture of the same bacilli in the blood of *Cardium norvegicum*.

(4) Filtered extract of the tissues of *Cardium norvegicum* which had been killed by heat and then minced in sea-water.

(5) The fresh blood of *Cardium norvegicum*.

(6) Sea-water.

It was assumed that a positive result was given when over twenty corpuscles were seen free in the tube, and a mass of agglutinated corpuscles was found at the mouth of the tube and extending a little way up it. On this assumption, positive results were given with the culture in broth (1), the culture in blood (3), and the extract of the tissues (4), and negative results with the fresh blood (5), and sea-water (6). Varying and inconclusive results were given with the sterile fish-broth (2). These conclusions represent the mean of a large number of experiments. The chief experimental errors, which are liable to cause false results, are due to employing capillary tubes which are rough at the open end; in this case the opening becomes rapidly closed by a mass of agglutinated

corpuscles. Another source of error consists in using tubes containing air at the closed end, when the fluid may be expelled, or blood sucked in, as a result of changes of temperature. Even when the tube is completely full, time must be allowed after sealing the end, for it to take up the room temperature. The water in which the animal is kept should also be at the room temperature, and the tube should never be touched with the hand, to avoid warming.

I consider that these experiments show that cultures of bacteria and extracts of dead tissues have a positive chemio-tactic attraction for the corpuscles.

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 11. ——— "On Green Oysters," 'Quart. Journ. Micr. Sci.,' vol. 26, 1886, p. 71.
 12. ——— "Phagocytes of Green Oysters," 'Nature,' vol. xlviii, 1893, p. 75.
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EXPLANATION OF PLATE 31,

Illustrating Mr. G. H. Drew's paper on "Some Points in the Physiology of Lamellibranch Blood-Corpuscles."

Fig. 1.—Finely granular eosinophil corpuscles in expansion and contraction. Stained with eosin and methylene blue. $\times 750$.

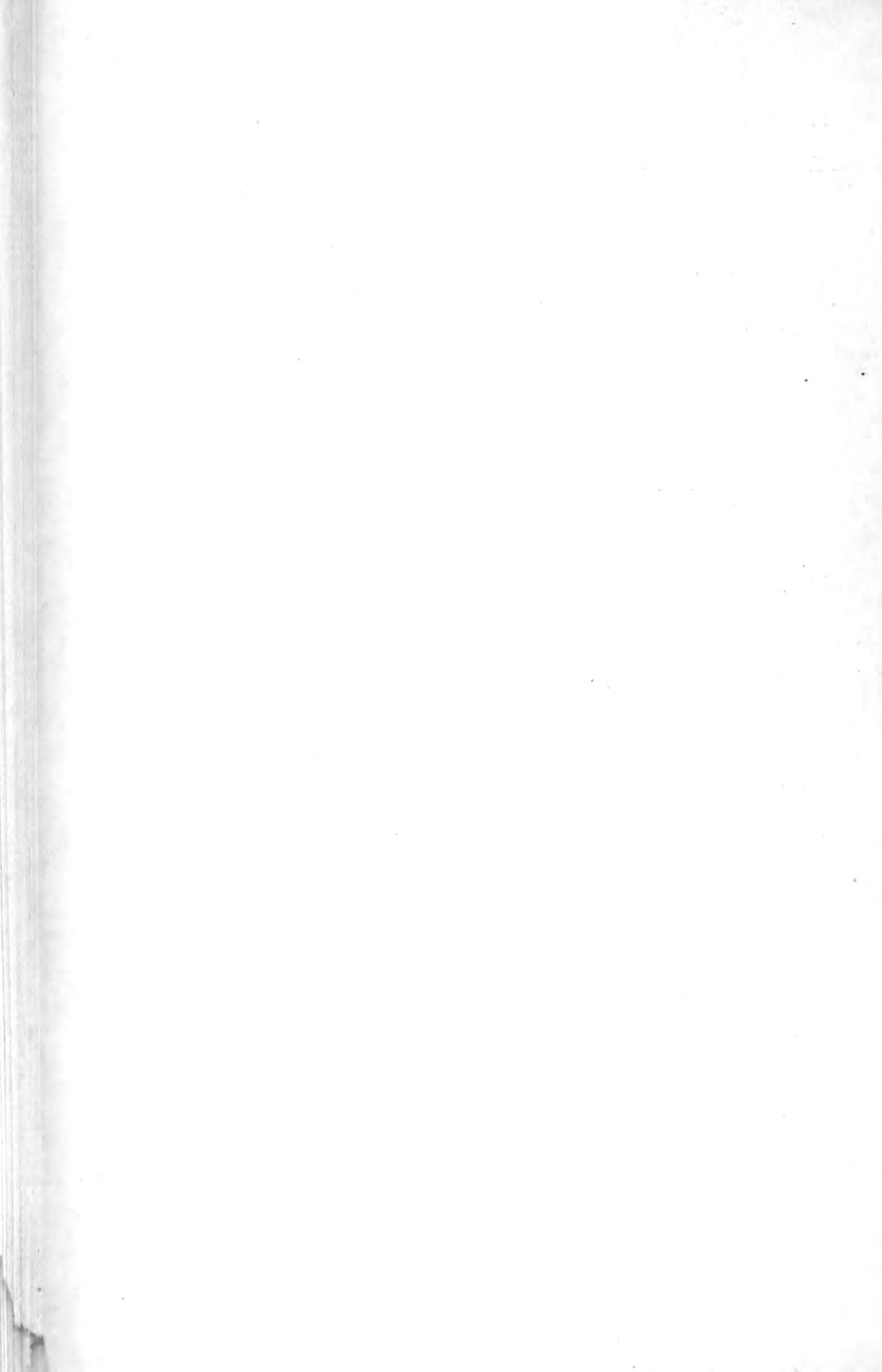
Fig. 2.—Coarsely granular eosinophil corpuscles in expansion and contraction. Stained with eosin and methylene blue. $\times 750$.

Fig. 3.—Basophil corpuscles in expansion and contraction. Stained with methylene blue. $\times 750$.

Fig. 4.—Agglutinated masses of corpuscles adherent to strands of cotton fibre, showing thin connecting bands of protoplasm. $\times 100$.

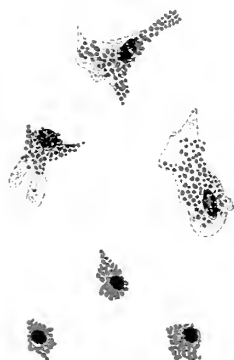
Fig. 5.—Later stage of fig. 4, showing thickening and contraction of the connecting bands of protoplasm. The cotton fibres have been drawn closer together by the contraction. $\times 100$.

Fig. 6.—Ingested bacteria in the corpuscles. $\times 750$.





1.



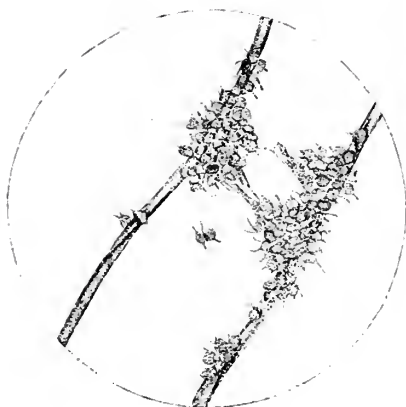
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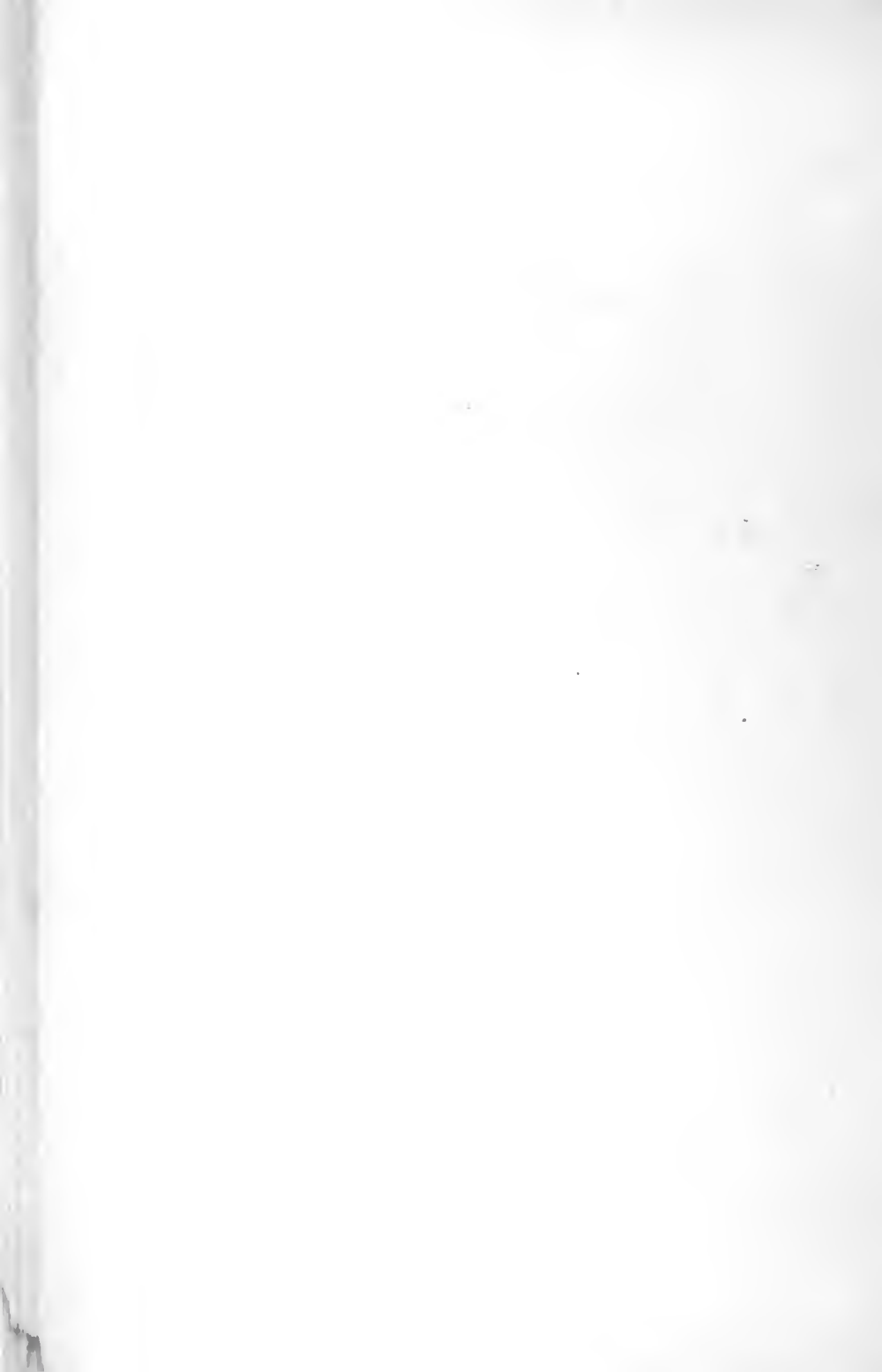
4.



5.



6.



Note on the Cytology of *Calothrix fusca*.

By

Dr. N. H. Swellengrebel,
Amsterdam.

With Plate 32.

WHILE studying the cytology of several *Trichobacterinae* I came across a representative of the group of *Cyanophyceae*, the study of which may cast perhaps some light on the question of relationship between *Cyanophyceae* and *Bacteria*.

I found *Calothrix fusca* in aquaria among large quantities of *Glœocapsa*; I never found it not associated with those algæ. It seems not impossible that a symbiotic relationship exists between those two algæ, a relationship which would be obviously beneficial to *Calothrix*, this species being more or less deprived of chlorophyll. This question, however, must remain for the while a mere hypothesis, because I was not able to study the question more thoroughly.

The dimensions of the cells are very variable. At the end of the cell-filaments the cells are rather short (from 3.6μ — 7.2μ), but lengthen towards the base (from 7.8μ — 10.8μ); their breadth is from 3.6μ — 7.2μ . The filaments which are enclosed in thick hyaline sheaths are pseudo-ramified. Each pseudo-ramification possesses at its base a heterocyst and some concave cells.

To study the cytological details the cell-filaments were fixed in Pfeiffer's solution, washed in alcohol 60 per cent., after the ordinary passages through alcohol embedded in

paraffin (52°), and cut in sections from 4—6 μ thick. The sections were washed in xylol and stained with iron-hematoxylin (Heidenhain).

I shall not enumerate here the different papers which have been published about the cytology of Cyanophyceæ. For reference to them the very complete works of Kohl,¹ Fischer,² and Guilliermond³ may be consulted. According to Guilliermond, who recently studied various representatives of this group, the central mass of stainable matter (the "central body" of Bütschli⁴) is composed of chromatin which is supported by an achromatic substratum of alveolar structure; the whole central body is to be regarded as a primordial nucleus, a view which was already held by Bütschli and his followers. A contrary opinion is upheld by A. Fischer, who does not believe in the nuclear nature of the central body; according to this author it represents only the central part of the cytoplasm free from chlorophyll. The chromatophil bodies within it consist of a peculiar hydrocarbon called "anabænine." His strongest argument against the chromatic nature of those granules consists in the fact that they are dissolved in water. It must be remembered, however, that this argument no longer holds good, since Oes⁵ showed that the chromosomes of *Spirogyra* are equally dissolved in water. Kohl (loc. cit.) showed that the chromatophil granules of the central body give all the characteristic reactions of true chromatin. I also have carefully examined the microchemical reactions of those granules, and have arrived at the same conclusion as Kohl's. I do not describe them here, since Kohl has done this in extenso. There cannot consequently be the least doubt that the granules within the central body

¹ Kohl, 'Über der Organisation und Physiologie der Cyanophyceen,' Jena, 1903.

² Fischer, 'Botan. Zeitung,' 1905.

³ Guilliermond, 'Revue général de botanique,' 1907.

⁴ Bütschli, 'Über die Bauder Cyanophyceen und Bacterien,' Leipzig, 1826.

⁵ Oes, 'Botan. Zeitung,' 1908.

consist of real chromatin. I also observed in the cells of *Calothrix fusca* the metachromatic granules (volutine granules); they always were found in the central body.

The cells of *Calothrix fusca* do not possess a well developed chromatophore, surrounding the central body, as is found in other members of the Cyanophyceæ. Often the cells are not at all green coloured, and when this is the case the green colour is diffusely spread throughout the cell without a well-marked differentiation between coloured and non-coloured cytoplasm. This is perhaps the reason why the central body of *Calothrix fusca* is never so compactly built as in other Cyanophyceæ, and why it becomes so easily diffuse.

The cytoplasm of the young cells (at the end of the filaments) contains few or no inclusions, and has an alveolar structure (Pl. 32, figs. 1—5) which is not always distinctly visible. The central body is generally of normal shape. It is formed of an achromatic substratum (which, however, stains more deeply than the surrounding cytoplasm), in which are embedded the chromatic granules and filaments. The achromatic substratum determines the form of the central body; this form is rather variable, often the central body is star-shaped, and resembles the same organ of *Tolypothrix lanata* described by Kohl (loc. cit.). The chromatin is not always distributed throughout the whole central body; often several parts of it are free from chromatin (Pl. 32, fig. 5). Such specimens are very favourable for the study of the relation between the achromatic substratum of the central body and the surrounding cytoplasm. Both have an alveolar structure, and by carefully examining the places where cytoplasm and achromatic substratum come together, one can often observe that the septa of the cytoplasmic alveoli are continued without interruption into those of the achromatic substratum, the only difference consisting in the different avidity with which stains are absorbed (Pl. 32, figs. 5, 6).

Cell division is performed in the ordinary way; in the middle of the cell an imperfect ring-shaped transverse mem-

brane is formed, which becomes afterwards closed. The central body divides by simple fissure, chromosome-like masses of chromatin not being found as is the case in other Cyanophyceæ (Kohl, Guilliermond).

Often it can be observed that the central body loses more and more its ordinary shape. It becomes elongated with more or less developed ramifications; often a slight curvature or zigzag form is to be observed (Pl. 32, figs. 2, 6, 7, 8 c). After carefully staining, one can always observe that the central body is normally formed by its two components, the chromatin and the chromatic substratum. In other specimens, however, the distinction between cytoplasm and achromatic substratum becomes more and more indistinct, and it is impossible at last to trace a distinction between the two (Pl. 32, figs. 8 d, e, 9). The chromatin is in such cases spread diffusely throughout the whole cell. The protoplasm of the latter is built after the ordinary pattern. The cells resemble very much those of some sporogenic bacteria, recently described by Guilliermond.¹

The dissolution of the central body described here was also observed by Guilliermond (loc. cit.) in *Scytonema cin-cinnatum*, but it occurred there only in old vacuolated cells, so it is highly probable that the dissolution was of a pathologic origin. This, however, cannot be the case in *Calothrix fusca*, as very young cells (at the ends of young cell-filaments) show already this phenomenon (Pl. 32, fig. 8). In grown-up cells there appear in the cytoplasm large hyaline granules. They surround the central body at first, and seem afterwards to invade the latter, so causing its dissolution. I was at first deluded by this phenomenon, thinking that the dissolution of the central body had a purely mechanical cause, due to an auto-destruction by the formation of the hyaline granules. But a closer observation made clear that the diffusion of chromatin is equally found in cells which are not provided with hyaline granules (Pl. 32, figs. 8, 9), so

¹ Guilliermond, 'Arch. f. Prot. kunde,' 1908.

the destruction of the central body has not a merely mechanical cause.

I vainly tried to make out the chemical nature of these hyaline granules. They are not identical with the "cyanophycinkörnchen" of the German authors, nor do they consist of fat. They are only a little to be stained with eosine and carbolic fuchsin, they are dissolved in diluted acids and in pepsine, not in diluted alkalies.

The changes in cell-structure in the microtome sections were controlled by the study of toto-stained preparations. After fixation the cells were placed on a cover-glass, and were stained and imbedded in the ordinary way. The normal central bodies had the same aspect as in the sections (Pl. 32, fig. 10). The beginning of dissolution of the central body was also very clearly to be seen (Pl. 32, fig. 12) in these preparations, also the cells with diffuse chromatin (Pl. 32, fig. 11). Generally the distinction between the protoplasma of the central body and the surrounding parts was not very clearly to be seen. Except this point the toto-preparations had the same value as the sections.

I will now shortly discuss the results of the observations described here. The normally built central body of *Calothrix fusca* contains chromatin granules imbedded in the alveoli of the plasma of the central body (the "achromatic substratum"). The latter is easily differentiated from the cytoplasm; the alveoli of the latter are the continuation of those of the achromatic substratum. I think therefore with Guilliermond that the central body of the *Cyanophyceæ* must be regarded as a primordial nucleus, the difference of cytoplasm and nuclear plasma already existing, but being not yet very distinctly marked. Under certain circumstances, unknown to me, the central body becomes at first irregularly shaped (in this stage the central body resembles strikingly the "diffuse nuclei" of *Opalinopsis* and *Foettingeria*), after which the difference between cytoplasm and nuclear plasma (s. achromatic substratum) disappears, and the chromatin granules are spread throughout the whole cell.

The stages with diffuse chromatin resemble very much the Bacteria with chromatic granules spread throughout the protoplasma. There is not yet differentiation between cytoplasm and nuclear plasma. Other forms of Bacteria, with their chromatin condensed into a more or less compact central mass (*Sphaerotilus* [Swellengrebel¹], *Bac. spirogyra* [Dobell²]), find their match in those forms of *Calothrix fusca*, where a well-marked differentiation between cytoplasm and nuclear plasma does not yet exist, but where the chromatin is no longer spread throughout the whole cell, but takes a central position (Pl. 32, fig. 8 c). The stage with a well developed central body is not yet found in the group of Bacteria.

Bütschli (loc. cit.) has already observed the resemblance of the structure of Cyanophyceæ and larger Bacteria, the latter showing a central agglomeration of chromatin suggesting a central body. I think that the stages with diffuse chromatin come much nearer to the structures described in Bacteria, and that these observations may aid to support the view concerning the relationship between Cyanophyceæ and Bacteria.

HYGIENIC INSTITUTE,
AMSTERDAM;
June, 1909.

EXPLANATION OF PLATE 32,

Illustrating Dr. N. H. Swellengrebel's paper "Note on the Cytology of *Calothrix fusca*."

(Drawings made under a Zeiss 2 mm. homog. oil immersion apochromatic comp. oc. 18.)

Figs. 1—9.—Sections from 4—6 μ thick.

Figs. 1 and 3.—Three cells showing a well-developed central body with differentiation into chromatin and achromatic substratum.

¹ Swellengrebel, 'C. R. Soc. de biol.,' Juin, 1908.

² C. C. Dobell, 'Quart. Journ. Micros. Science,' vol. 53, May, 1909.

FIGS. 2 and 4.—Central body in the act of becoming diffuse.

FIG. 5.—Showing the star-shaped achromatic part of the central body.

FIG. 6.—Idem. Achromatic part zigzag shaped.

FIG. 7.—As fig. 5, but achromatic substratum no longer visible.

FIG. 8.—Showing the different stages of dissociation of the central body.

a, b. Normal central body.

c. Central body become diffuse. Achromatic substratum no longer differentiated.

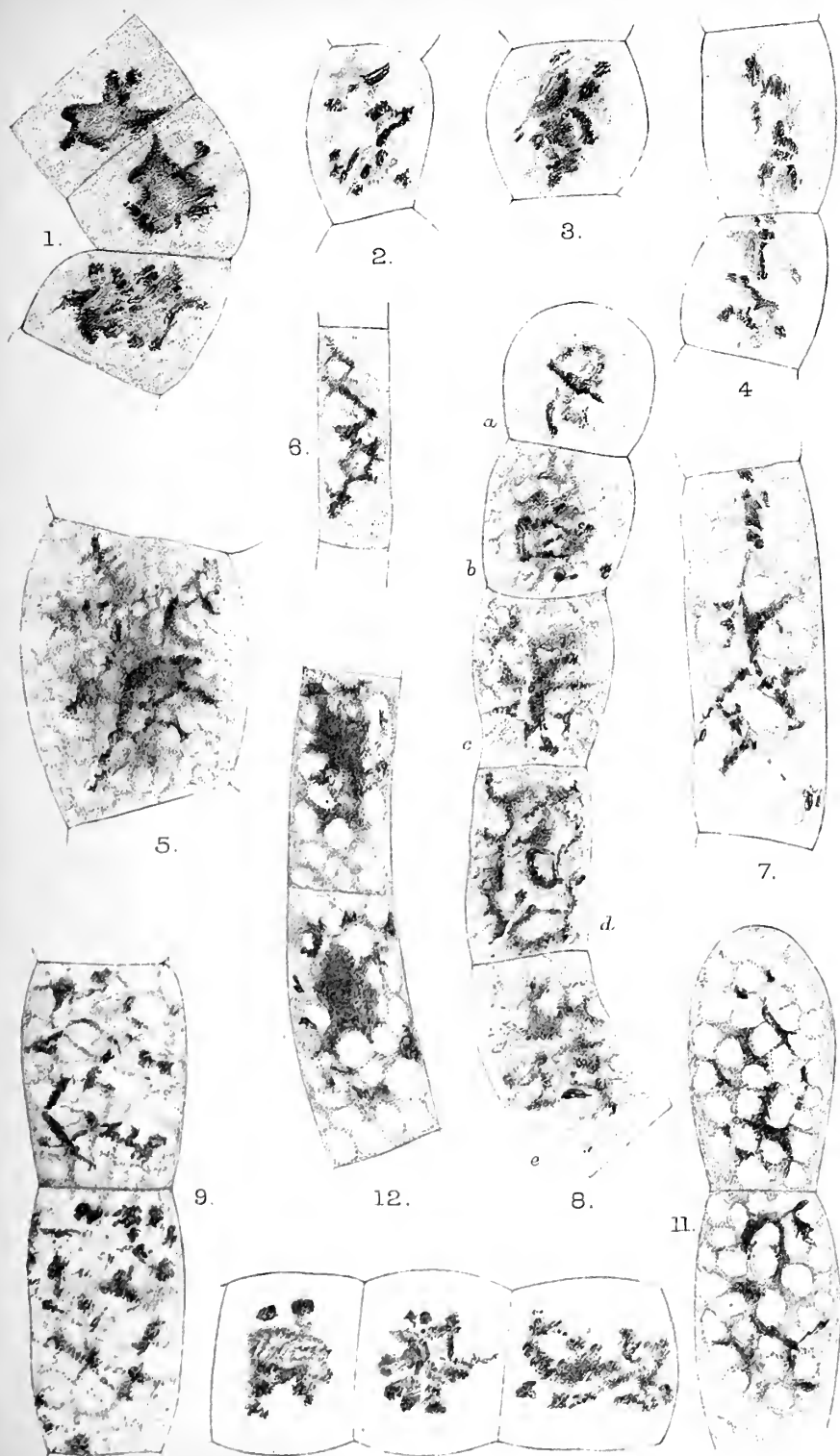
d, e. Complete dissolution of the central body. Chromatin in the form of granules and filaments spread throughout the protoplasm.

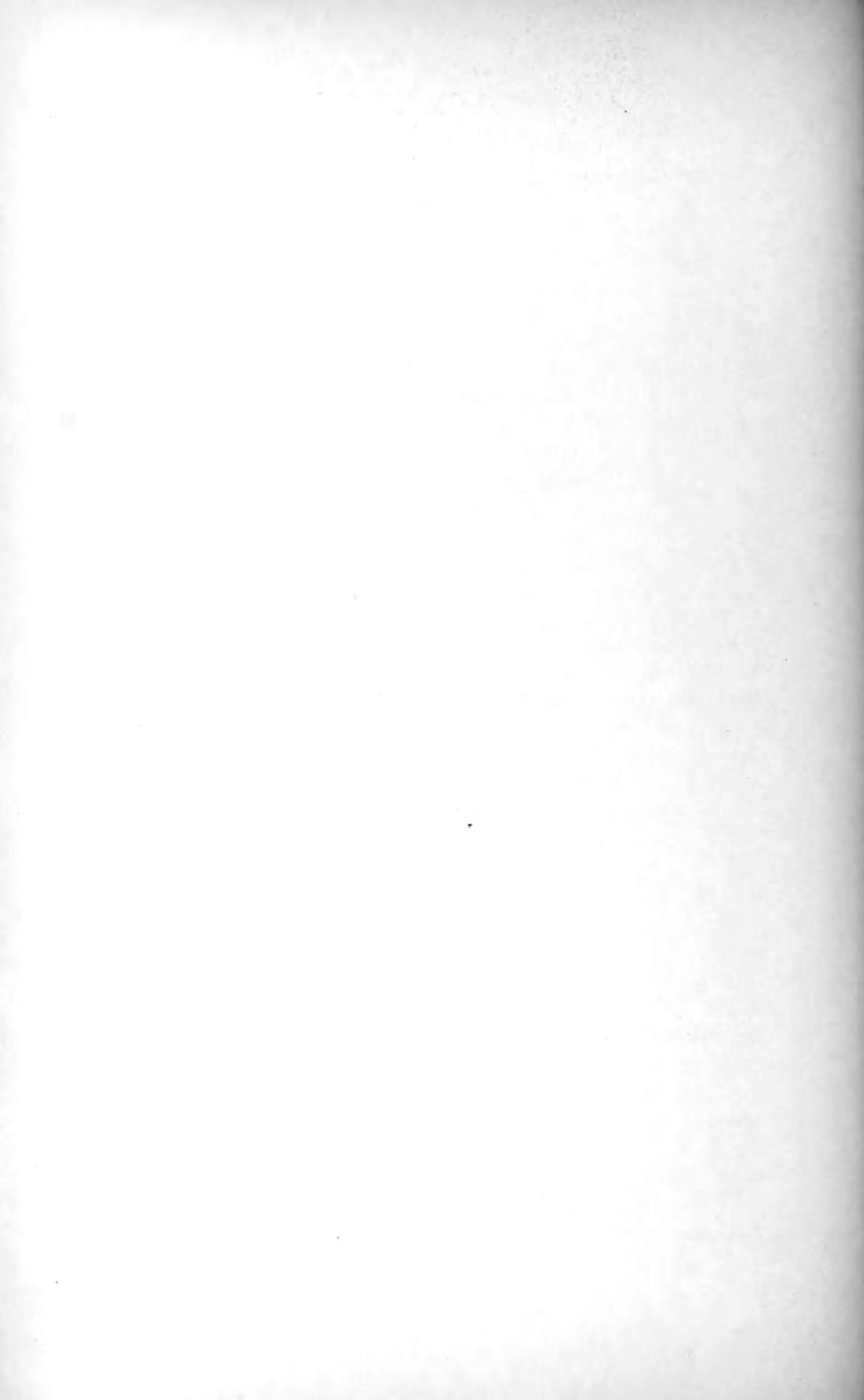
FIG. 9.—Same stages as fig. 8*d* and 8*e*.

FIGS. 10—12.—Preparations of cell-filaments stained in toto.

The figures show the same peculiarities as the microtome sections.







Tropidonotus and the "Archenteric Knot" of Ornithorhynchus.

By

Richard Assheton, M.A.

With Plate 33.

IN my paper in a recent number of this Journal, in which I discussed Professor Hubrecht's memoir upon the ontogenetic phases of mammalia, I referred to the peculiar condition of the egg of Ornithorhynchus as described by Wilson and Hill in the 'Philosophical Transactions of the Royal Society,' vol. cxcix, and the interpretation placed by them thereon.

A remarkable spot found by these authors and named by them "primitive or archenteric knot" exists at an early stage of the blastocyst of Ornithorhynchus, a stage before the upraising of the neural folds, at some distance in front of the primitive streak which is present in a form perfectly typical of mammalia.

The whole blastocyst at this period is "occupied by mainly fluid contents," the more solid yolk of the previous stage (6 mm. in diameter) having become partly disintegrated by absorption of fluid from the uterus by this time, when the diameter of the blastocyst has attained 10 mm.

Wilson and Hill regarded this spot as representing an early stage in the development of the archenteron, and attempted to identify it with the "Hensen knot" of the later period. Considerable gaps exist in the material, so that they were not able to trace accurately either the origin or the fate of this structure.

I ventured to suggest that this spot had nothing to do with archenteron formation, and that it might be a quite erroneous conclusion to identify it with the anterior end of the primitive streak of later times. I argued at some length to show from their description that their interpretation is not tenable. As an alternative I offered the suggestion that the spot in question is the morphological lower pole of the egg, and gave figures illustrating a comparison between *Ornithorhynchus* as described by Wilson and Hill and a Sauropsidan, such as the sparrow, postulating a complete growth round by the edge of the blastoderm at an earlier stage in the Prototherian than in the Sauropsidan egg in correlation with a smaller quantity of yolk. The fact that there is a very well-marked eccentricity—I mean that this spot is not diametrically opposite to the centre of the “embryo” in *Ornithorhynchus* as it is in the sparrow, but as it is not in the rabbit—did not seem to me to be a serious objection.

Since making this criticism I have come across a series of sections which I cut through the lower pole of the egg of *Tropidonotus natrix* some years ago, but which I had forgotten—at a stage represented by the outline drawing (Pl. 33, fig. 1).

I find I have also in toto the same spot indicating the coalesced edges of the blastoderm of another egg of the same snake, and the appearances of these specimens are such as to add very considerably to the degree of probability that the suggestion I made will turn out to have been well founded.

The resemblance between the drawings of sections taken through the centre of this area (fig. 2) and Wilson and Hill's text-fig. 4 (p. 51) of their section through the archenteric knot of *Ornithorhynchus* is most striking. It must be remembered that at this stage in the snake's egg the “blastocyst cavity” is still nearly filled with yolk, which is not shown in my drawing, as the loose yolk has been all washed off during the preparation of the specimen, this loose yolk corresponding to the “tolerably large remainder of the original yolk as a more or less coherent mass, lying free within

the cavity of the vesicle" of the Ornithorhynchus egg of 10-12 mm. in diameter (p. 42).

Fig. 2 is the 239th section of the series of 440 sections passing through the spot, and so is nearly central.

This shows that the lower pole of the egg of *Tropidonotus*, after the yolk has become completely enveloped by the edge of the blastoderm, consists of the following parts: On the outside there is the epiblast (*ep.*), a thin layer, except at the centre where its free edge has concentrated forming the dense mass of cells (*c. p.*). Beneath this there is, towards the periphery, the hypoblast (*hy.*), consisting of a reticulum containing many yolk-spherules (*y. g.*), and small nuclei on the surface next the epiblast. This hypoblast layer is seen to be much thicker near the centre of the figure, and a few more deeply placed nuclei may be found. This is comparable to that part which, in the chick, was termed by Balfour "the germinal wall" (*g. w.*). Beneath the plug of epiblast (*c. p.*) is a deeply staining and very finely granular material (*y.*), which is the just-covered superficial layer of the lower pole of the yolk. Even as it is, the resemblance between this structure and the so-called "archenteric knot of Ornithorhynchus" is sufficiently marked to cause one to view with suspicion the interpretation placed upon that spot by Wilson and Hill. But if we imagine a slightly more advanced condition, if we imagine the coalescence of the germinal wall, either by a gradual closing of the ring or by a differentiation of the small remaining piece of pure yolk into germinal wall, the resemblance between the two structures would be even more marked.

In fig. 3 I have drawn a diagram from this figure and adopted the same method of indicating the layers as Wilson and Hill use in their text-fig. 4, p. 51, using the same lettering but attaching to them my interpretation. Thus *ect.* is the epiblast or ectoderm in each case, *ent.* the hypoblast.

The epiblast (*ect.*) is obviously in continuation with the cell-plug (*c. p.*), which is seen in *Tropidonotus* to be the thickened coalesced margin of the epiblast of the blastoderm

The part which projects above the surface, the actual cell-plug, is probably partly due to a growth of the epiblast cells after the coalescence of the margin, as I have found certainly one mitotic figure within this mass. In some sections, e. g. fig. 4, the distinction into "cell-plug" (*c. p.*) and "central more cellular zone" (*c. z.*) is still more marked than it is in fig. 2.

The part called by Wilson and Hill the "marginal or cortical zone of the knot tissue" (*m. z.*) is represented by the germinal wall part of the hypoblast of *Tropidonotus*, which, like the author's marginal zone, can be well described as showing "coarsely reticular, indefinite and feebly staining characters," and being "poor in nuclei which are chiefly met with near its entodermal aspect."

Fig. 4 is another section of the same specimen which passes through a little cleft still remaining, which shows the relations of the thin epiblast to the thickened rim very clearly.

It is difficult to resist the conclusion that a condition similar to this has given rise to the state of affairs in *Ornithorhynchus*, described by Wilson and Hill and indicated in their text-fig. 5, p. 52, and figs. 9 and 10, pl. 4.

If my comparison is a correct one, the archenteric knot of *Ornithorhynchus* with its anterior and posterior lips of the blastopore and its "commencement of true archenteric invagination" may be dismissed, and another stumbling-block will be removed from the path of the student of mammalian embryology.

As regards the question of the eccentricity of this spot in *Ornithorhynchus* I have no further evidence to bring forward. I have no note on this point and cannot recollect whether the point of coalescence of the blastoderm is diametrically opposite to the mid-dorsal surface or not. The egg of *Tropidonotus* is long. Even if the point of coalescence in the snake's egg is exactly opposite to the upper pole I do not think that it invalidates in any way the argument from the morphological point of view.

Whether, if this is the correct interpretation, it supports

or opposes the view that the Prototherian egg is derived from an egg of the Sauropsidan type may be open to question. There can be very little doubt that, if such has been its origin, there would be a condition as suggested. But it would be hazardous to assert that those are the only circumstances under which such a condition could have arisen.

One can only say that the condition in all essential features is identical with that of the Sauropsidan type, and is totally unlike that of Amphibia, Teleosteans, Dipnoi, Elasmobranchs, or Cyclostomes. To my mind it appears to form strong evidence in confirmation of the validity of the association of the Reptilia, Aves, and Mammalia in one distinct group—the Amniota.

Fig. 5 shows the cellular character of the cell-plug, the thin general layer of epiblast continuous with the thickened margin, and the hypoblast and yolk-spherules, many disintegrated, forming the germinal wall or marginal zone of Wilson and Hill.

AROS,
ISLE OF MULL,
August, 1909.

PAPERS REFERRED TO IN THE TEXT.

1. Assheton, R.—"Professor Hubrecht's Paper on the Ontogenetic Phases of Mammalian Development: An Appreciation and Respectful Criticism," 'Quart. Journ. Micr. Sci.,' vol. 54, 1909.
2. Wilson, J. T., and Hill, J. P.—"Primitive Knot and Early Gastrulation Cavity Coexisting with Independent Primitive Streak in Ornithorhynchus," 'Proc. Roy. Soc. London,' vol. lxxi, 1903.
3. ———— "Observations on the Development of Ornithorhynchus," 'Phil. Trans. Roy. Soc. London,' ser. B, vol. cxcix, 1907.

EXPLANATION OF PLATE 33,

Illustrating Mr. Richard Assheton's paper on "Tropidonotus and the ' Archenteric Knot ' of *Ornithorhynchus*."

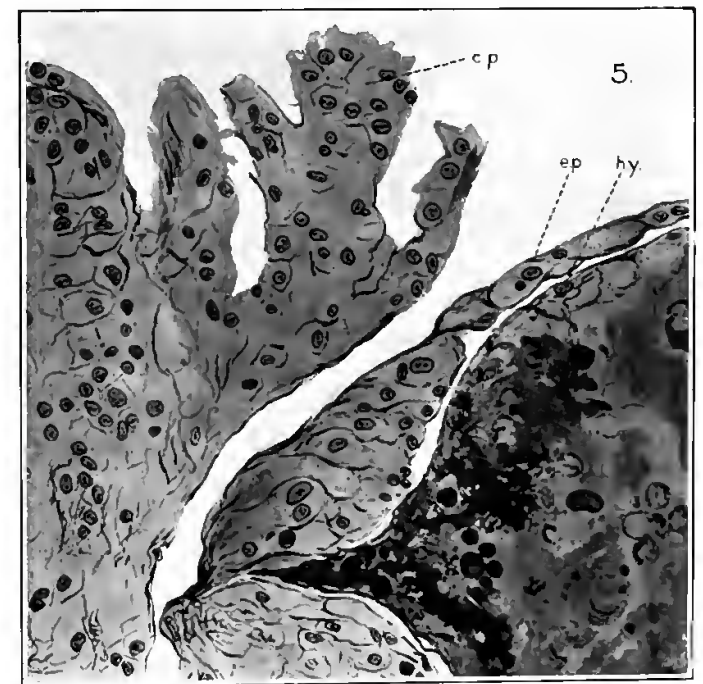
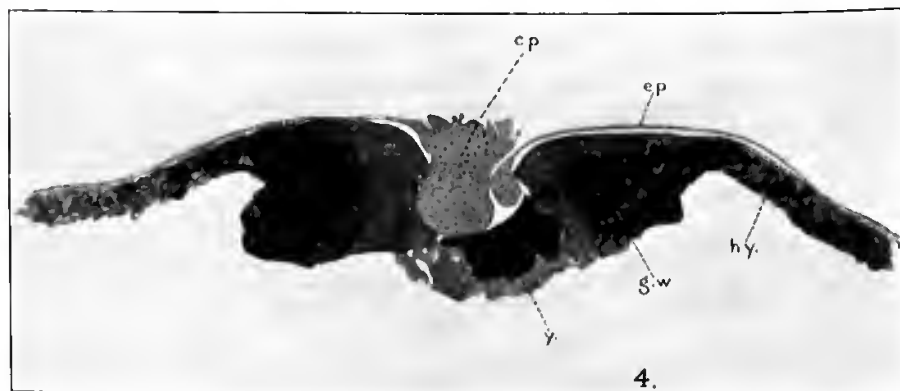
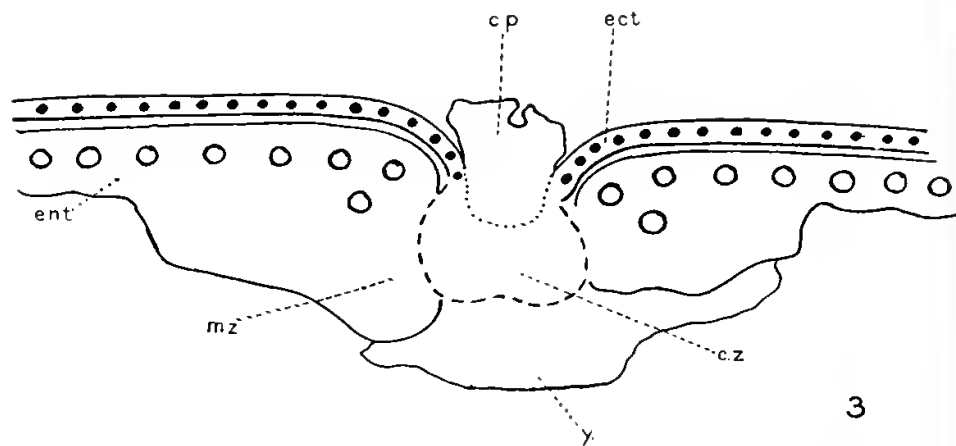
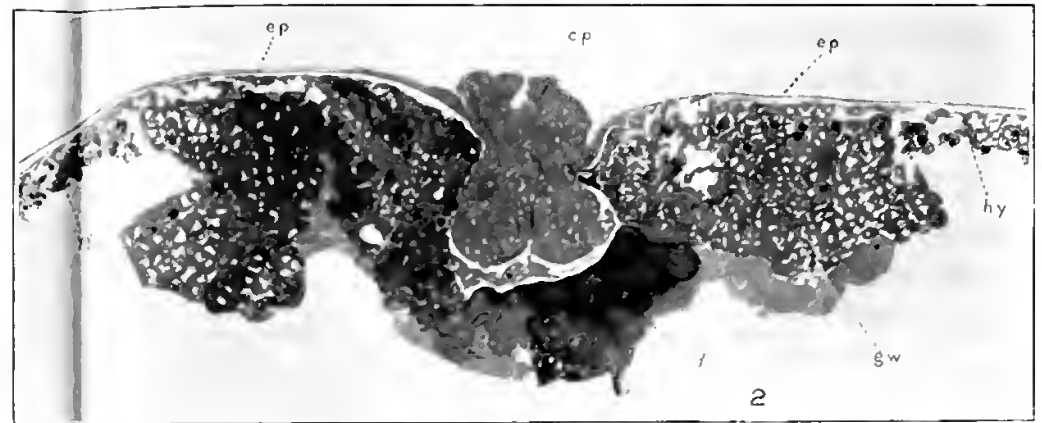
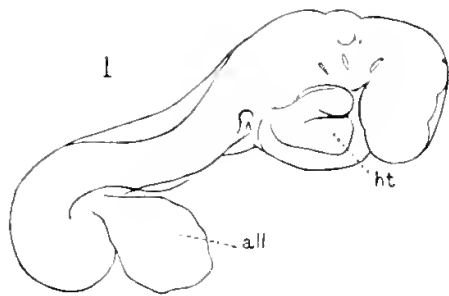
Fig. 1.—Outline figure of *Tropidonotus* embryo, showing three gill-clefts, allantois, etc. *all.* Allantois. *ht.* Heart.

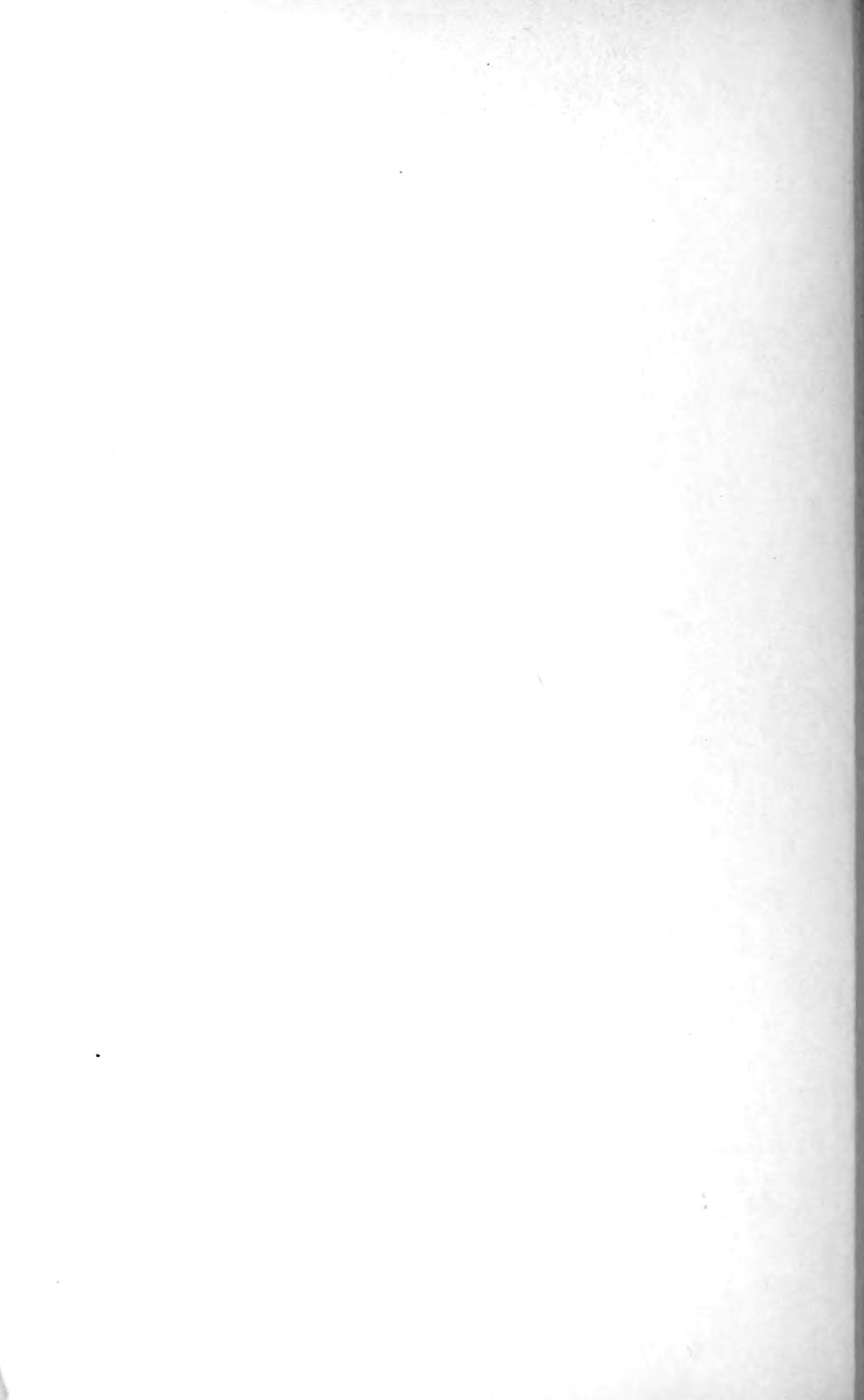
Fig. 2.—Transverse section through the central region of the lower pole of the egg of *Tropidonotus* after complete envelopment of the yolk by the blastoderm of the stage of fig. 1. The loose yolk has been washed away, leaving hypoblast and germinal wall as a thickening round the fused edge of the epiblast. *c. p.* Heaped-up rim of coalesced epiblast. *e. p.* Epiblast. *hy.* Hypoblast with much yolk. *g. w.* Germinal wall. *y.* Pure yolk not yet covered by, or converted into germinal wall.

Fig. 3.—Diagram of the above, lettered and drawn after the manner of Wilson and Hill's text-fig. 4. with which it should be compared. *c. p.* Cell-plug of ectoderm. *ect.* Ectoderm. *ent.* Endoderm. *c. z.* (Central zone) part of central plug of ectoderm obviously in continuity with the ectoderm. *m. z.* (Marginal or cortical zone) = germinal wall, or hypoblasts and yolk. *y.* Yolk.

Fig. 4.—Another section of the same series of transverse sections through the central region of the lower pole of *Tropidonotus*. The section passes through a little cleft still remaining between the edges of the coalesced blastoderm rim. *c. p.* Plug of cells derived from the coalesced rim of the blastoderm. *e. p.* Epiblast. *hy.* Hypoblast. *g. w.* Germinal wall. *y.* Yolk.

Fig. 5.—A portion of the section shown in fig. 4, to illustrate the cellular character of the "cell-plug" (*c. p.*) (coalesced blastoderm edge), and *e. p.* epiblast, *hy.* germinal wall (hypoblast).





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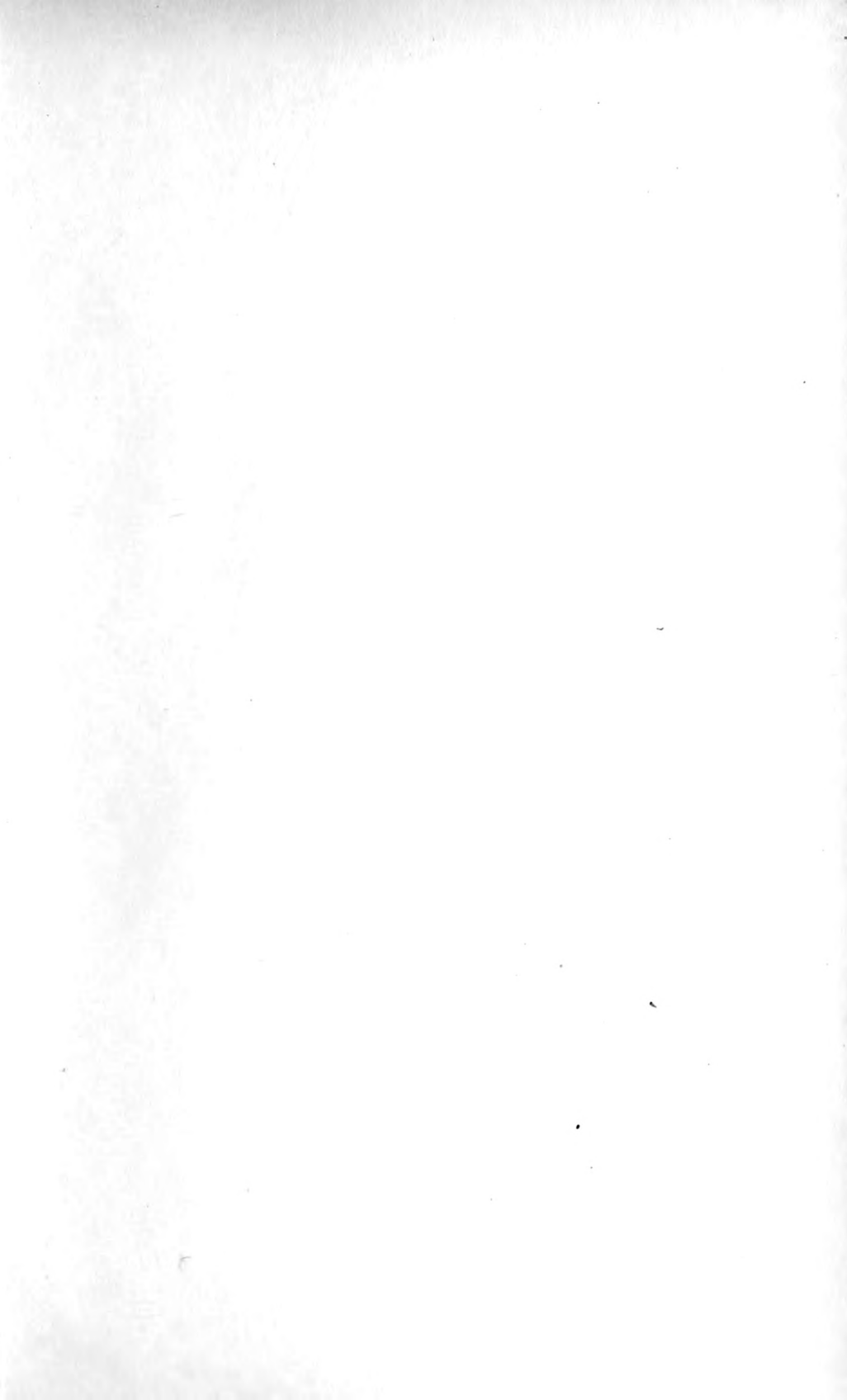
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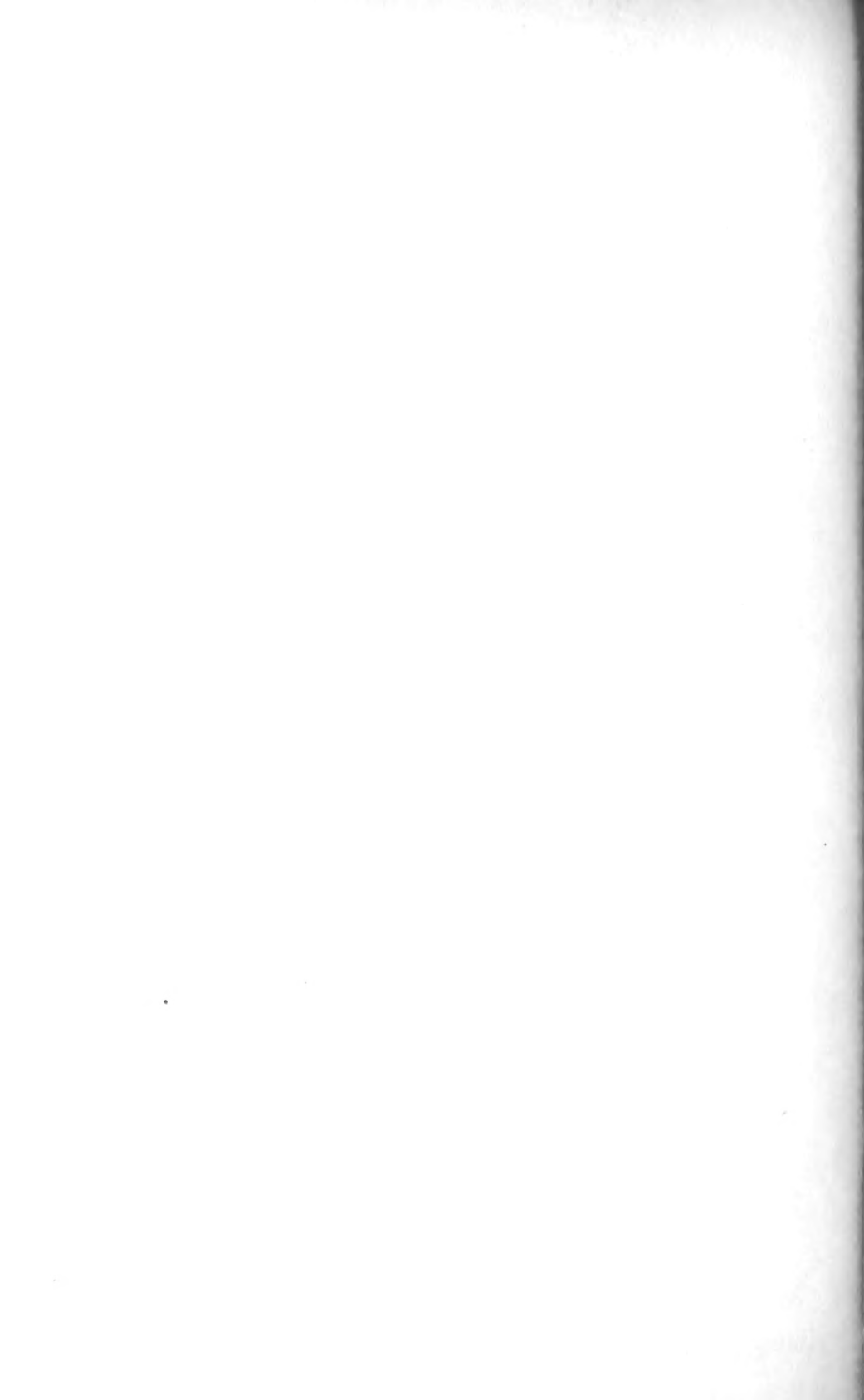
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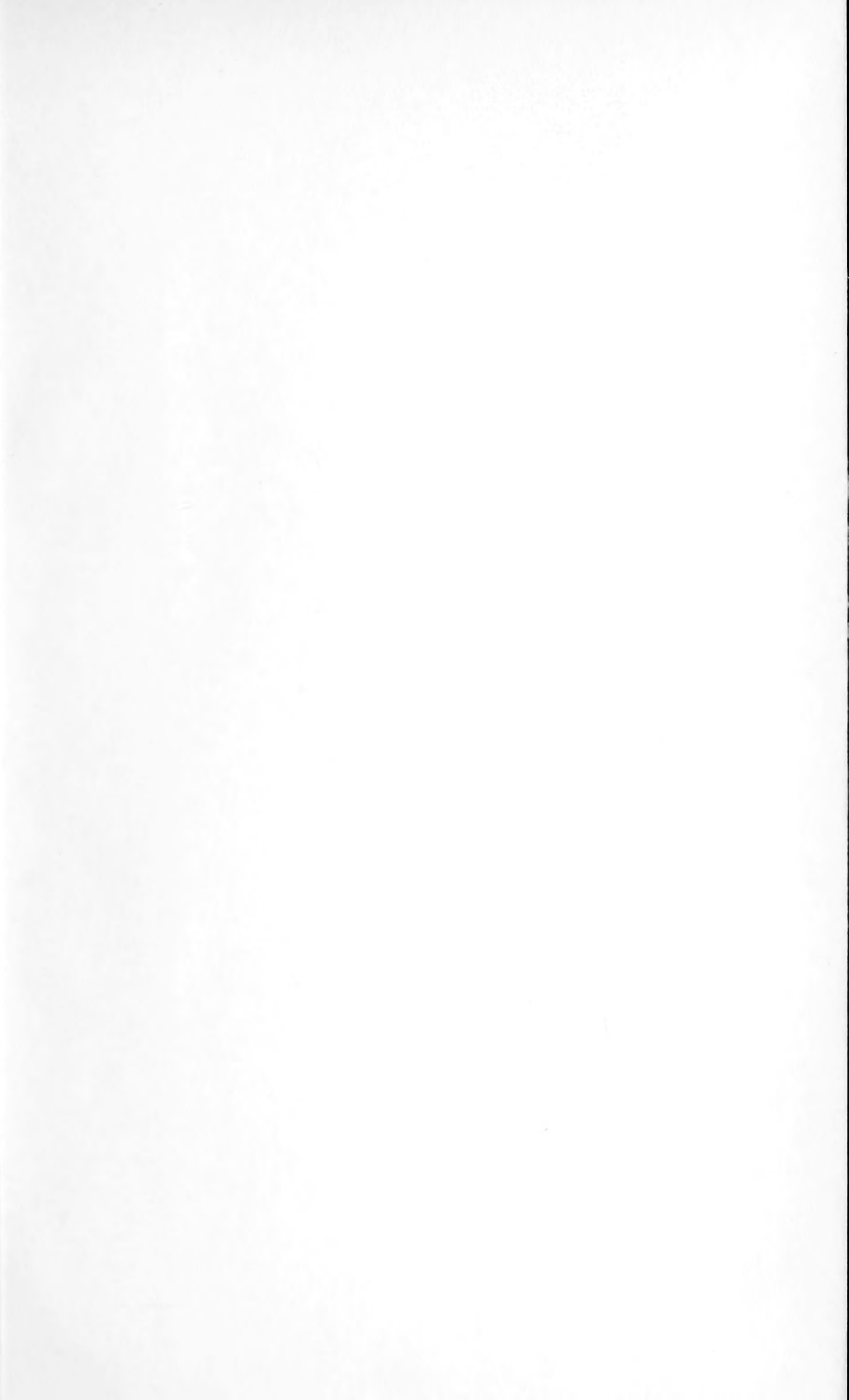
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